# Emotional Reactions after Event Learning

## A Rift between Implicit and Explicit Conditioned Valence in Humans Pain Relief Learning


Inaugural Dissertation

zur Erlangung der Doktorwürde der Philosophischen Fakultät II
der Julius-Maximilians-Universität Würzburg

vorgelegt von Marta Andreatta

Würzburg, 23. Dezember 2009

Erste Gutachter: Prof. Dr. Paul Pauli

Zweite Gutachter: Prof. Dr. Alessandro Angrilli

Tag des Kolloquiums: 18.06.2010

*„Wo aber Gefahr ist,*
*wächst das Rettende auch.“*

F. Hölderlin

This work would have never been possible without help, collaboration and support by many people.
I would like to thank briefly:

**Prof. Dr. Paul Pauli** for his support and crucial advice concerning the work. I have learned a lot from him. And I have felt his constant presence during the GK events really important. Furthermore, I really enjoyed staying at his department considering that I should have stayed just for six months and it turned out into three-year time. Sincerely thanks.

Not less important and helpful was **Dr. Andreas Mühlberger**. Especially, I thank him for precious advices delivered during these years, for time dedicated for designing my research and for helping in the analyses.

How can I not thank the two people from whom the idea for my PhD has started? **Prof. Dr. Bertram Gerber** and **Dr. Ayse Yarali.** At the begging, we have really taken a leap into the unknown trying to transfer the outcomes from fruit flies to humans. However, nowadays that every thought worked out so well, it is even more satisfactory! Thank you both for the hints.

I want also give a special thank to **Prof. Dr. Alessandro Angrilli** who boosted me toward the research. Research that I really like and for which have grown a deep passion.

A particular thank should go to **Stefan Schulz**. The coffee breaks were fundamental moments during the working day. I thank him also for letting my 'Italian temperament' free and calming me down to tackle situations more professionally.

It would have been really boring to spend the most of the day (and not only!!) at the University without my **colleagues and the nice staff of the Department**! For exchanging ideas and opinions, for relaxing and funny moments during the movie evenings, the barbecues in the department garden, thank you all of you!

The experience I had would be much less great without all the colleagues-friends of the **GK people**. Despite our different personalities and backgrounds, we have been through so many events, working together and still having fun. I really enjoyed doing the PhD together with

you all. A special thank to **Anne, Saskia**, **Sarah** and **Johannes** for the funny evenings spent together.

And the last, but not at all the least, my best friends, **Ilka Unsold** and **Melanie Bauer**. *Liebe Ilka und liebe Mel, ohne Eure Freundschaft hätte ich nie geschafft, vier Jahre in Deutschland zu halten. Ihr wart meine Unterstützung in den schwierigen Momenten, mein Spaß in den lustigen Momenten und meine wertvollen Lehrerinnen sowohl der Deutschsprache als auch der Deutschkultur. Herzlichen Dank!*

# Content

**Abstract** ..... 9

**Zusammenfassung** ..... 11

**1. Introduction** ..... 14

**1.1. Theoretical Context** ..... 18

*1.1.1. The Two-Process Learning Theory (Rescorla, 1967)* ..... *18*

*1.1.2. The Opponent-Process Theory (Solomon, 1980)* ..... *20*

*1.1.3. The Sometimes Opponent-Process Theory (Wagner, 1981)* ..... *24*

**1.2. Affective Associative Learning** ..... 24

*1.2.1. Fear Conditioning* ..... *25*

*1.2.2. Appetitive Conditioning* ..... *28*

*1.2.3. Event Timing* ..... *30*

**1.3. Neural Correlates of Associative Learning** ..... 34

*1.3.1. Neural Correlates of Fear Conditioning* ..... *34*

*1.3.2. Neural Correlates of Appetitive Conditioning* ..... *40*

*1.3.3. Neural Projections of the Defensive and Appetitive Systems* ..... *45*

**1.4. Goal and Hypothesis** ..... 46

**2. The Modulation of the Startle Response Induced by Event Learning** ..... 48

**2.1. Forward and Backward Delay Conditioning (Experiment 1)** ..... 53

**2.1.1. Method** ..... 53

*2.1.1.1. Participants* ..... *53*

*2.1.1.2. Stimulus Material* ..... *53*

*2.1.1.3. Procedure* ..... *54*

*2.1.1.4. Physiological Recording and Data Reduction* ..... *55*

*2.1.1.5. Data Analysis* ..... *55*

**2.1.2. Results** ..... 57

*2.1.2.1. Subjective Ratings* ..... *57*

*2.1.2.2. Startle Amplitude* ..... *58*

*2.1.2.3. Contingency Awareness of the CS-US Association* ..... *58*

**2.1.3. Discussion** ..... 60

**2.2. Forward and Backward Conditioning, Delay vs. Trace (Experiment 2)** ..... 62
**2.2.1. Method** ..... 63
*2.2.1.1. Participants* ..... *63*
*2.2.1.2. Procedure* ..... *63*
*2.2.1.3. Data Analysis* ..... *63*
**2.2.2. Results** ..... 64
*2.2.2.1. Subjective Ratings* ..... *64*
*2.2.2.2. Startle Amplitude* ..... *65*
*2.2.2.3. Contingency Awareness of the CS-US Association* ..... *66*
**2.2.3. Discussion** ..... 66
**2.3. General Discussion** ..... 69

**3. The Neural Circuits Underlying Event Learning** ..... 73
**3.1. Method** ..... 77
*3.1.1. Participants* ..... *77*
*3.1.2. Stimulus Material* ..... *77*
*3.1.3. Procedure* ..... *77*
*3.1.4. Magnetic Image Resonance* ..... *78*
*3.1.5. Image Preprocessing and Statistical Analysis* ..... *79*
**3.2. Result** ..... 81
*3.2.1. Subjective Ratings* ..... *81*
*3.2.2. Functional Neuroimaging* ..... *82*
*3.2.3. Contingency Awareness of CS-US Association* ..... *85*
**3.3. Discussion** ..... 85

**4. The Attentional Processes Underlying Event Learning** ..... 89
**4.1. Method** ..... 92
*4.1.1. Participants* ..... *92*
*4.1.2. Stimulus Material* ..... *92*
*4.1.3. Procedure* ..... *93*
*4.1.4. Physiological Recording and Data Reduction* ..... *94*
*4.1.5. Data Analysis* ..... *95*
**4.2. Results** ..... 96
*4.2.1. Subjective Ratings* ..... *96*

*4.2.2. Contingency Awareness of CS-US Association* ..... *97*
*4.2.3. Steady-State Visual Evoked Potentials* ..... *98*
**4.3. Discussion** ..... 100

**5. General Discussion** ..... 103
**5.1. Outlook** ..... 109

**6. Glossary** ..... 114

**7. Reference** ..... 117

**8. Appendix** ..... 133
**8.1. Sketch of the Paradigm** ..... 133
**8.2. Informed Consent Form** ..... 134
*8.2.1. Informed Consent Form of the Study 1* ..... *134*
*8.2.2. Informed Consent Form of the Study 2* ..... *137*
*8.2.3. Informed Consent Form of the Study 3* ..... *139*
**8.3. Questionnaire** ..... 141
*8.3.1. Experiment Sign-up Sheet: Study 1 and Study 2* ..... *141*
*8.3.2. Experiment Sign-up Sheet: Study 3* ..... *142*
*8.3.3. STAI, German Version* ..... *144*
*8.3.4. STAI, Italian Version* ..... *146*
*8.3.5. The Edinburg Inventory* ..... *150*
**8.4. Pain Threshold Procedure** ..... 151
*8.4.1. Sheet for the Pain Threshold for the Study 1 and the Study 2* ..... *151*
*8.4.2. Sheet for the Pain Threshold for the Study 3* ..... *152*
**8.5. Curriculum Vitae** ..... 153
**8.6. Publications** ..... 155
*8.6.1. Papers* ..... *155*
*8.6.2. Conference Proceedings* ..... *155*

## Abstract

In order to survive, organisms avoid threats and seek rewards. Classical conditioning is a simple model to explain how animals and humans learn associations between events that allow them to predict threats and rewards efficiently. In the classical conditioning paradigm, a neutral stimulus is paired with a biologically significant event (the unconditioned stimulus – US). In virtue of this association, the neutral stimulus acquires affective motivational properties, and becomes a conditioned stimulus (CS+). Defensive responses emerge for pairings with an aversive US (e.g., pain), and appetitive responses emerge for pairing with an appetitive event (e.g., reward). It has been observed that animals avoid a CS+ when it precedes an aversive US during a training phase (*CS+ → US*; *forward conditioning*); whereas they approach a CS+ when it follows an aversive US during the training phase (*US → CS+*; *backward conditioning*). These findings indicate that the CS+ acquires aversive properties after a forward conditioning, whereas acquires appetitive properties after a backward conditioning.

It is thus of interest whether event timing also modulates conditioned responses in such an opponent fashion in humans, who are capable of explicit cognition about the associations. For this purpose, four experiments were conducted in which a discriminative conditioning was applied in groups of participants that only differed in the temporal sequence between CS+ onset and US onset (i.e., the interstimulus interval – ISI). During the acquisition phase (*conditioning*), two simple geometrical shapes were presented as conditioned stimuli. One shape (CS+) was always associated with a mild painful electric shock (i.e., the aversive US) and the other one (CS-) was never associated with the shock. In a between-subjects design, participants underwent either forward or backward conditioning. During the test phase (*extinction*), emotional responses to CS+ and CS- were tested and the US was never presented. Additionally, a novel neutral shape (NEW) was presented as control stimulus. To assess cognitive components, participants had to rate both the valence (the degree of unpleasantness or pleasantness) and the arousal (the degree of calmness or excitation) associated with the shapes before and after conditioning.

In the first study, startle responses, an ancestral defensive reflex consisting of a fast twitch of facial and body muscles evoked by sudden and intense stimuli, was measured as an index of stimulus implicit valence. Startle amplitude was potentiated in the presence of the forward CS+ whilst attenuated in the presence of the backward CS+. Respectively, the former response indicates an implicit negative valence of the CS+ and an activation of the defensive

system; the latter indicated an implicit positive valence of the CS+ and an activation of the appetitive system.

In the second study, the blood-oxygen level dependent (BOLD) response was measured by means of functional magnetic resonance imaging (fMRI) to investigate neural responses after event learning. Stronger amygdala activation in response to forward CS+ and stronger striatum activation in response to backward CS+ were found in comparison to CS-. These results support the notion that the defensive motivational system is activated after forward conditioning since the amygdala plays a crucial role in fear acquisition and expression. Whilst the appetitive motivational system is activated after backward conditioning since the striatum plays a crucial role in reward processing.

In the third study, attentional processes underlying event learning were observed by means of steady-state visual evoked potentials (ssVEPs). This study showed that both forward and backward CS+ caught attentional resources. More specifically, ssVEP amplitude was higher during the last seconds of forward CS+ that is just before the US, but during the first seconds of backward CS+ that is just after the US. Supposedly, attentional processes were located at the most informative part of CS+ in respect to the US.

Participants of all three studies rated both forward and backward CS+ more negative and arousing compared to the CS-. This indicated that event timing did not influence verbal reports similarly as the neural and behavioral responses indicating a dissociation between the explicit and implicit responses.

Accordingly, dual process theories propose that human behavior is determined by the output of two systems: (1) an impulsive implicit system that works on associative principles, and (2) a reflective explicit system that functions on the basis of knowledge about facts and values. Most importantly, these two systems can operate in a synergic or antagonistic fashion. Hence, the three studies of this thesis congruently suggest that the impulsive and the reflective systems act after backward association in an antagonistic fashion.

In sum, event timing may turn punishment into reward in humans even though they subjectively rate the stimulus associated with aversive events as being aversive. This dissociation might contribute to understand psychiatric disorders, like anxiety disorders or drug addiction.

## Zusammenfassung

Organismen vermeiden Gefahren und streben nach Belohnungen, um zu überleben. Klassische Konditionierung ist ein einfaches Model, das erklärt, wie Tiere und Menschen Ereignisse in Verbindung bringen. Dieses Lernen ermöglicht Lebewesen Gefahr oder Belohnung direkt vorherzusehen. Normalerweise besteht das Konditionierungsparadigma aus der Präsentation eines neutralen Stimulus zusammen mit einem biologisch bedeutsamen Event (der unkonditionierte Stimulus – US). Aufgrund dieser Assoziation erwirbt der neutrale Stimulus affektive Eigenschaften und wird dann konditionierter Stimulus (CS+) genannt. Wenn der CS+ mit Schmerz während der Trainingsphase assoziiert wird, leitet er eine defensive Reaktion, wie z.B. Vermeidung ein. Wenn der CS+ mit einer Belohnung assoziiert wird, leitet er eine appetitive Reaktion, wie z.B. Annäherungsreaktionen ein. Interessanterweise haben Tierstudien gezeigt, dass ein konditionierter Stimulus vermieden wurde, wenn er einem aversiven US in der Trainingsphase vorausgegangen war (*CS+ → US*; *Vorwärtskonditionierung*). Das deutet darauf hin, dass der CS+ aversive Eigenschaften erlangt hat. Jedoch führte ein konditionierter Stimulus zu einer Annäherung, wenn er in der Trainingsphase auf einen aversiven US folgt (*US → CS+*; *Rückwärtskonditionierung*). Das deutet darauf hin, dass der CS+ appetitive Eigenschaften erlangt hat.

Kann das Event Timing sowohl aversive als auch appetitive konditionierten Reaktionen auch bei Menschen auslösen, die zu Kognitionen bezüglich der Assoziationen fähig sind? Um diese Fragestellung zu beantworten, wurden vier Studien durchgeführt. Die Studien hatten den gleichen Ablauf, variiert wurde nur die Zeit zwischen CS+ und US (das Interstimulusintervall – ISI – ist als das Zeitintervall zwischen dem Onset des CS+ und dem Onset des US definiert). Während der Akquisitionsphase (*Konditionierung*) wurden, zwei einfache geometrische Figuren als konditionierte Stimuli dargeboten. Eine geometrische Figur (der CS+) war immer mit einem leichten schmerzhaften elektrischen Reiz (der aversive US) assoziiert; die andere Figur (der CS-) war nie mit dem elektrischen Reiz assoziiert. In einem *between-subjects* Design wurde entweder eine Vorwärtskonditionierung oder eine Rückwärtskonditionierung durchgeführt. Während der Testsphase (*Extinktion*) wurden CS+ und CS- präsentiert sowie zusätzlich eine neue neutrale geometrische Figur präsentiert, die als Kontrollstimulus fungierte; der US wurde in dieser Phase nie dargeboten. Vor und nach der Konditionierung wurden die Probanden sowohl bezüglich der Valenz (bzw. Unangenehmheit und Angenehmheit) als auch der Erregung (bzw. Ruhe und Aufregung) hinsichtlich der geometrischen Figuren befragt.

In der ersten Studie wurde der Schreckreflex (Startle Reflex) als Maß für die implizite Valenz der Stimuli gemessen. Der Schreckreflex ist eine defensive Urreaktion, die aus einem Muskelzucken des Gesichts und des Körpers besteht. Dieser Reflex ist durch plötzliche und intensive visuelle, taktile oder akustische Reize evoziert. Einerseits war die Amplitude des Startles bei der Anwesenheit des vorwärts CS+ potenziert und das deutet daraufhin, dass der CS+ eine implizite negative Valenz nach der Vorwärtskonditionierung erworben hat. Anderseits war die Amplitude des Startles bei der Anwesenheit des rückwärts CS+ abgeschwächt, was darauf hin deutet, dass der CS+ nach der Rückwärtskonditionierung eine implizite positive Valenz erworben hat.

In der zweiten Studie wurde die oxygenierte Bloodsresponse (BOLD) mit funktioneller Magnetresonanztomographie (fMRI) erhoben, um neuronale Korrelate des Event-Timings zu erfassen. Eine stärkere Aktivierung wurde in der Amygdala in Erwiderung auf den vorwärts CS+ und im Striatum in Erwiderung auf den rückwärts CS+ gefunden. Zum Einen entspricht dies einer Aktivierung des Defensive Motivational Systems, da die Amygdala eine wichtige Rolle beim Angstexpression und Angstakquisition hat. Deshalb wurde der vorwärts CS+ als aversiv betrachtet. Zum Anderen entspricht dies einer Aktivierung des Appetitive Motivational System, da das Striatum eine wichtige Rolle bei Belohnung hat. Deshalb wurde der rückwärts CS+ als appetitiv betrachtet.

In der dritten Studie wurden Aufmerksamkeitsprozesse beim Event-Timing näher beleuchtet, indem steady-state visuelle evozierte Potentiale (ssVEP) gemessen wurden. Sowohl der vorwärts CS+ als auch der rückwärts CS+ zog Aufmerksamkeit auf sich. Dennoch war die Amplitude der ssVEP großer während der letzen Sekunden des vorwärts CS+, d.h. direkt vor dem aversiven US. Die Amplitude der ssVEP war aber größer während der ersten Sekunden des rückwärts CS+, d.h. kurz nach dem aversiven US. Vermutlich wird die Aufmerksamkeit auf den hinsichtlich des aversiven US informativsten Teil des CS+.

Alle Probanden der drei Studien haben den vorwärts CS+ und den rückwärts CS+ negativer und erregender als den Kontrollstimulus beurteilt. Daher werden die expliziten Ratings vom Event-Timing nicht beeinflusst. Bemerkenswert ist die Dissoziation zwischen den subjektiven Ratings und den physiologischen Reaktionen. Nach der Dual-Prozess Theorie werden die Verhaltensreaktionen des Menschen von zwei Systemen determiniert: einem impulsiv impliziten System, das auf assoziativen Prinzipien beruht, und einem reflektiv expliziten System, das auf der Kenntnis über Fakten und Werte basiert. Wichtig ist, dass die zwei Systeme auf synergetische oder antagonistische Weise agieren können. Folglich könnte

es sein, dass das impulsive und das reflektive System nach der Rückwärtskonditionierung antagonistisch arbeiten.

Zusammen deuten die vorliegenden Studien daraufhin, dass Event-Timing eine Bestrafung in eine Belohnung umwandeln kann, aber die Probanden erleben den Stimulus assoziiert mit einem aversiven Event als negativ. Diese Dissoziation könnte zum Verständnis der psychiatrischen Störungen wie z.B. Angststörungen oder Drogenabhängigkeit beitragen.

# 1. Introduction

To survive, an organism avoids threats and seeks rewards. Hence, animals and humans adapt themselves to imminent biologically significant events as for example threats or food.

Pavlovian or classic conditioning is a widely used paradigm to understand brain mechanisms underlying both emotional learning and memory and provides a valuable method for studying how animals and humans learn to associate and predict events (Kim, & Jung, 2006). Classical conditioning is involved in abnormal behavior such as drug addiction and anxiety disorders (Bouton, Barlow, & Mineka, 2001).

In a typical study, a neutral stimulus is paired with a primary reinforcer (called unconditioned stimulus; US) like an electric shock (aversive reinforcer) or a sucrose solution (appetitive reinforcer). After few pairings, the presence of the neutral stimulus alone elicits a range of emotional reactions such as freezing, salivation, changes in heart rate and blood pressure, increased startle responses, stress hormone release and approach behavior according on the qualities of the reinforcer. In other words, the neutral stimulus, which is now called conditioned stimulus (CS), predicts the occurrence of the US and as a consequence the organism reacts to it with defensive or appetitive behaviors appropriately. These acquired emotional reactions to CS are then called conditioned responses (CR). Hence, classical conditioning is a form of associative learning involving linkage between a neutral stimulus (CS) and a stimulus with high intrinsic behavioral significance (US) that enable the organisms to predict events, therefore assuring its survival.

Organism responses to external threats or to appetitive cues eliciting two motivational systems that have opponent properties: The defensive and the appetitive system (Lang, Bradley, & Cuthbert, 1998). Accordingly, the two motivational systems operate in an opponent manner that is the more active the defensive system is the less active the appetitive system is and vice versa. The presence of a biologically salient aversive event elicits the defensive system and all the corresponding responses depending on the proximity of such an event; whereas the presence of a biologically salient appetitive event elicits the appetitive system and all the corresponding responses. For example, as soon as the organism detects a threat, its vigilance towards it is increased, but the organism keeps on doing what it was doing. With the increasing of the proximity of the threat, the ongoing activity is stopped in order to make the resources available for escape or for fighting when the escape is not possible. Then, as soon as the threat is over, the fear response is reduced and the organism returns to its daily activity. In their hypothesis Lang and colleagues (1998) consider emotions as action dispositions since they motivate the organism to nurturance responses, to sexual

approach, to fight or to flight based on the external cues. Referring this hypothesis to the classical conditioning, a neutral stimulus after associative learning signals the proximity of a biologically salient cue (US) eliciting those memory associations and action programs that are linked to the engaged motivational system. That means that when a neutral stimulus is associated with an aversive event, the presence of such a stimulus alone "primes" the defensive motivational system, whereas when it is associated with an appetitive event, CS primes the appetitive motivational system. And this priming allows the organism to react promptly, assuring its survival.

Beside the apparent simplicity of associative learning, three factors are crucial in making associations, namely the contiguity, the contingency and the predictability. Contiguity refers to the temporal proximity of a CS and the US and is a central concept, but not sufficient to produce conditioning on its own. In fact, the capability of CS+ to appropriately predict the presence or the absence of US also seems to be important. The contingency refers to the probability of US in the presence of the CS as opposed to its absence that is CS gives information about US. In order to induce "excitatory" responses to the conditioned stimulus, US needs to occur more frequently in the presence of such stimulus than in its absence (Rescorla, 1988). The third factor for making associations is the predictability. With the term predictability is meant the discrepancy between an actually received US (e.g., a reward) and its prediction (i.e., the expected reward). Such a discrepancy between what it is expected and what it is got is termed prediction error (PE). Learning ($\Delta V$, associative strength) is proportional to the prediction error ($\lambda$-V) and reaches its asymptote when the prediction error approach zero after several learning trials. Thus, organisms tend to adapt the degree of their effort according to the magnitude of reward they expect. When they get a smaller reward than they expect (i.e., the prediction error results as negative), then organism will tend not to repeat that behavior, whereas when they got the reward they expected or even a bigger one (i.e., the prediction error results as positive), then such a behavior is preferred. All three factors, the contiguity, the contingency and the prediction error, must to be satisfied for learning to occur.

Theories of associative learning have been concerned with these factors and have tried to explain the interplay among contiguity, contingency and prediction error. However, some theories focused on the role of attention in such interplay, but differ in the rules they proposed for determining whether or not attention is paid to a stimulus. Other theories focus on the nature of the association that is formed (either aversive or appetitive), but differ as to whether this association is regarded as elemental or hierarchical. Furthermore, less concern has been given to the temporal factors that certainly contribute to whether conditioning occurs. In an

elegant review, Hamm and Weike (2005) explained the two-level account for classical conditioning in humans. Their model makes it pretty clear that associative learning involves two levels in humans namely, an automatic, non-conscious level and a cognitive, conscious level. The former does not required cognitive processing of CS supporting the idea that the defensive system can be activated by the direct thalamus-amygdala connection. The latter reflects the cognitive and conscious processing of CS as well as the relationship between events. In this case it has been suggested that the hippocampus might be the crucial cortical structure. Evidence of this two-level learning was found using delay and trace conditioning. Delay conditioning implicates that CS and US offset overlapped, whereas trace conditioning implicates that there is a temporal gap between CS offset and US onset. As is mentioned above, these two kinds of conditioning outline two completely different associative mechanisms. In fact, delay conditioning did not involve explicit knowledge of the association between CS and the aversive US to induce a potentiated conditioned response. Hence, it could be automatic learning. While, because conditioned fear responses are elicited after trace conditioning, the individual need to be aware of the association. Hence, such learning is not automatic, but let us say cognitive. This suggests that the temporal sequence of the events is also a fundamental factor in determining the relationship between CS and US.

Additionally, an interesting and recent animal study (Tanimoto, Heisenberg, & Gerber, 2004) drew particular attention to event timing suggesting that punishment may turn into reward. The authors observed that the association with an aversive US induced opponent CRs that are avoidance of or approach to CS in *drosophila melanogaster* depending on the stimuli temporal sequence. In other words, when the neutral stimulus (i.e., an odor; CS) *followed* a painful electric shock (the aversive US) which is defined forward conditioning, fruit flies avoided the conditioned odor in a testing phase; whereas if the odor *preceded* the shock (backward conditioning), flies approached it suggesting that such odor became pleasant or at least safety (Figure 1.1.). Curiously, human research does not take serious account of event timing. There is increasingly evidence showing how delay and trace conditioning involve different processes (Tabbert, Stark, Kirsch, & Vaitl, 2006), but there is little research into the processes involved in forward and backward conditioning. Here, I looked into the role of timing in making association between events in humans. In particular, I wondered if humans also learn opponent reactions (i.e., avoidance and approach) to a CS as the result of the temporal sequence between CS and an aversive US like the fruit flies showed. Furthermore, I wondered whether such opponent reactions are underlined by the cortical structures of the defensive motivational system (e.g., amygdala) and/or the appetitive motivational system

(e.g., striatum) respectively. To reach this goal I used the classical conditioning paradigm comparing forward and backward associations between a neutral and an aversive stimulus.

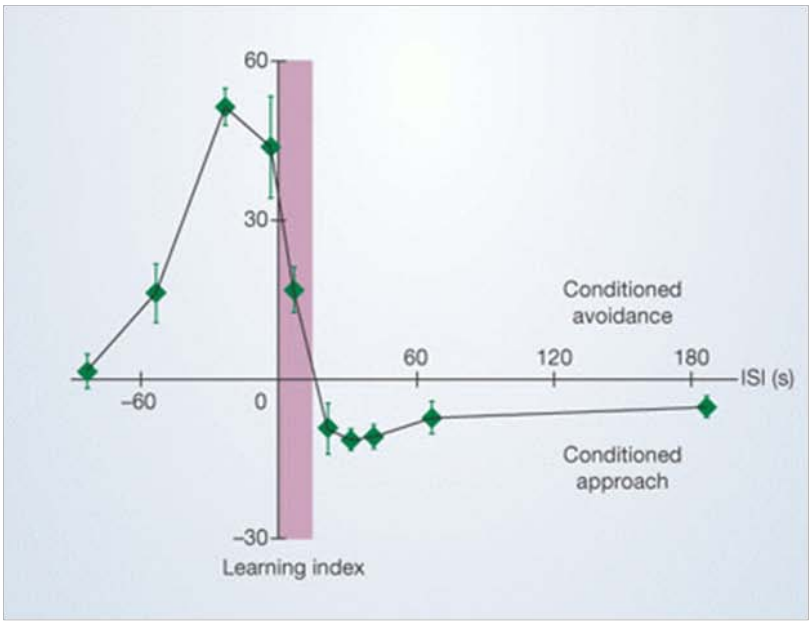



*Figure 1.1.* Results of the study with fruit flies (Tanimoto et al., 2004) comparing conditioned responses after forward and backward conditioning.

The values on the x-axis represent the time in seconds. The green dots represent the conditioned odor (CS+) and the pink shading is the US (an electric shock). The positive values on the y-axis indicate avoidance and the negative values approach. The ISI between the onset of the conditioned odor and the shock varied between groups of fruit flies. Flies avoided the odor which preceded the electric shock during the acquisition phase (left panel); whereas flies approached the odor which followed the shock during the acquisition phase (right panel). "*Conditioned behavior was significant for the group with ISI of -23 s, -3 s, 32 s and 42 s (P = < 0.005; one sample t-test against zero).*" (from the figure legend).

Firstly, I present the theoretical background regarding associative learning. Secondly, I characterize the neural correlate involved in associative learning. And subsequently, I explain the three studies I conducted to test the role of event timing in humans. The terms classical conditioning, Pavlovian conditioning, differential conditioning and the associative learning are used here as synonyms and refer to a paradigm in which a stimulus (CS+) is associated with the unconditioned stimulus (US), whereas a second stimulus (CS-) is not. According to the valence of the US, I refer to aversive conditioning if the US is an aversive/painful event and to appetitive conditioning when the US is an appetitive/rewarding event. Since, however,

in fruit flies the association with an aversive US provoked two kinds of reactions, I label the associations, in which US follows CS, forward conditioning or punishment learning since such associative learning (should) induces defensive responses; whereas I label the associations, in which US precedes CS, backward conditioning or relief learning since such associative learning (should) induces appetitive responses.

## 1.1. Theoretical Context

### *1.1.1. Two-Process Learning Theory (Rescorla, 1967)*

The temporal contiguity and the repetition of two events are historically considered the sources of all sensory knowledge. Thus, if two experiences occurred repeatedly and closely together in time, they are likely to be associated and one becomes the signal of the coming of the other. Starting from this observation, (Rescorla, & Solomon, 1967) highlighted that the *relation* between the neutral stimulus and the biological significance (i.e., salience) of the events is crucial in the development of a conditioned reaction. Thus, the strength of a conditioned reaction depends upon the strength of the connection between the representations of CS and US. Hence, the CS will induce CRs only if it is a good predictor of US. Consistently, Rescorla, and Solomon (1967) showed that an equivalent number of tone-shock pairings might or might not result in eliciting a conditioned response. Using an elegant paradigm, Rescorla (1968) showed that only when a stimulus was consistently associated with a shock, it elicited fear conditioned reactions. Whereas when the CS/US contiguity was held constant and additionally US occurred in absence of the CS, the CS did not elicit conditioned responses afterwards. At first glance, these findings appeared troublesome. Based on these results, Rescorla supposed that other factors should affect the associative learning. In fact, he defined the contingency as the probability of the US in the presence of the CS (p[US|CS]) minus the probability of the US in the absence of CS (p[US|noCS] and argued that the contingency serves as direct measure of the predictability of the CS in regard to the US. Only when p[US|CS] is higher than p[US|noCS], does CS elicit CR otherwise no CR would be shown. Therefore, the contingency measures nothing but the strength of the association and accordingly the subject shows the correspondent response.

By means of the behavioral responses, Rescorla differentiated between *conditioned excitation* and *conditioned inhibition*. In other words, both the conditioned excitation and the conditioned inhibition reflect the change in behavior resulting from the contingency between the CS and the US that is depending on the strength of the association. The most common example for the conditioned excitation is the increase of salivation in a dog in the presence of

a neutral sound, which was previously presented in sequence with food. Parallel attempts to specify conditioned inhibition have not been laid out so sharply. Moreover, a conditioned inhibitor is specified only in terms of a change in behavior referred to the conditioned excitation. That is, the stimulus associated with appetitive US (e.g., food) becomes an excitor and therefore salivation is induced just by the presence of CS. Whilst a stimulus becomes an inhibitor when it comes to control a tendency opposite to that of the conditioned excitor does using the same US. If the presence of an excitor induces an increase in the CR, i.e. increase of the salivation, the presence of an inhibitor induces a reduction of such CR, i.e. reduction of the salivation. In term of Rescorla's model, when the probability of US is higher in the presence of CS than in its absence (p[US|CS] > p[US|noCS]), then CS becomes a conditioned excitor and CRs are shown. If, however, the probability of US is higher in the absence of CS than in its presence (p[US|noCS] > p[US|CS]), then CS becomes a conditioned inhibitor and the CRs are reduced. Processing this information allows the organism to prepare itself to respond appropriately to the situation. Considering differential conditioning in which one stimulus is associated with US (CS+) and the other is not (CS-), CS- comes to control a tendency opposite to that of CS+. Indeed, when CS+ has a positive CS-US contingency and consequently signals US likelihood it then elicits CR; CS-, on the other hand, has a negative CS-US contingency, thereby signaling the absence of US and inhibiting CR. In both cases the organism gets information about the imminence of an appetitive/aversive event. Interestingly, the contingency seems to govern the behavioral expression in animals and humans in a similar way. The evidence of the conditioned inhibitory properties of CS- comes mainly from Konorski's laboratory. In their classic experiment, Konorski and Szwejkowska (1959) measured the amount of salivation (the appetitive response) conditioned by a food (the appetitive US) and the magnitude of a leg-flexion response (the aversive response) conditioned by a shock (the aversive US). They found that appetitive responses were increased in the presence of the CS+ associated with food and aversive responses were increased in the presence of the CS+ associated with the shock. Interestingly, Konorski and Szwejkowska were also able to observe that prior appetitive conditioning to a CS has an inhibitory or antagonist effect on the defensive response to the same CS when it is subsequently associated with an aversive US. It appears, hence, that the conditioned excitor of one affective value has an inhibitory effect on a response established with a reinforcer of opposite affective value. Further studies (Dickinson, & Dearing, 1979; Rescorla, 1968) suggested that an appetitive CS has both an excitatory effect on the appetitive system and an

inhibitory effect on the defensive system. Besides this, an aversive CS has an excitatory effect on the defensive system and an inhibitory effect on the appetitive system.

An important application of the appetitive-aversive interaction was provided by Seligman and Binik (1971) and its safety signal theory. Seligman correctly observed that when CS+ reliably predicts an aversive US, the probability of US given CS+ is 1.0 (i.e., positive contingency) and the conditioned defensive responses to that CS+ are elicited. However, the absence of the CS+ perfectly predicts the absence of that aversive US and conditioned defensive response are inhibited. Safety is defined as the periods free of aversive US, hence according to Seligman's hypothesis, fear is predicted by the presence of CS+ and safety by the absence of CS+. In other words, the presence and the absence of CS+ becomes a signal for how to predict US. However, if an organism has a history of uncontrollable aversive events that is it is unable to predict aversive US correctly and promptly, such an organism remains chronically in fear. It has been demonstrated (Mineka, & Zinbarg, 2006) that exposure to uncontrollable and unpredictable aversive events is important for the etiology and maintenance of generalized anxiety disorders (GAD). Furthermore, associative learning may also explain the development of other anxiety disorders such as phobias, posttraumatic stress disorder (PTSD) or panic disorders (Mineka, & Oehlberg, 2008; Mineka, & Zinbarg, 2006).

### *1.1.2. Opponent-Process Theory (Solomon, 1980)*

The *opponent-process theory* (Solomon, 1980) assumes that two opponent processes are initiated by the stimulus appearance. Thus, the presentation of either an appetitive or aversive stimulus is followed by a primary reaction (State A), which is characterized by the same affective state as the releaser that is hedonic or aversive respectively. It then declines slowly while the stimulus is still present approaching a relatively steady level. When the stimulation is terminated, there is a quick phasic decrease in the affect until the baseline is crossed and then a new, contrasting affective state (State B) emerges. Such a State B quickly approaches a peak and then slowly decrease in magnitude until the original affective baseline is reestablished (Figure 1.2.). These two states are characterized by an affective contrast, affective habituation and affective withdrawal. Affective contrast means opposite emotional valence of the two states, i.e. one is aversive and the other appetitive. An example with an aversive reinforcer comes from the reactions of military parachutists. During their first free-fall, before the parachute opens, military parachutists may experience terror, they may yell, pupils dilated, eyes bulging, bodies curled forward and stiff, heart racing and breathing irregular. After they land safely, they may walk around with stunned and stony-faced

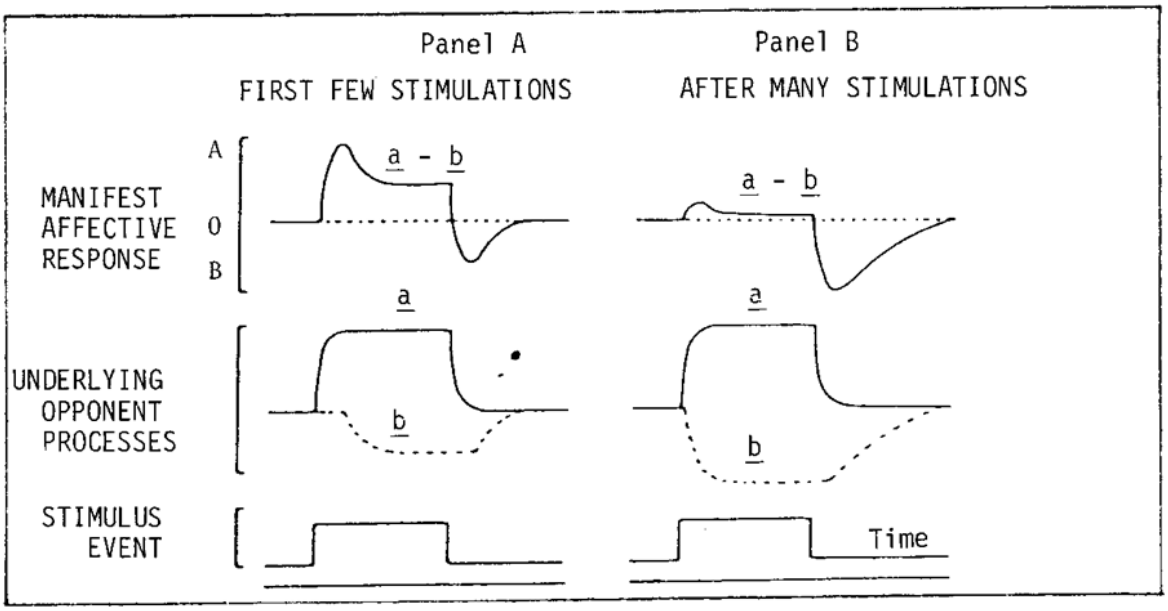



*Figure 1.2.* The opponent-process theory (Solomon, 1980).

The 'Stimulus Event' (on the bottom) indicates the onset and offset of an unconditioned stimulus. The 'Manifest Affective Response' (on the top) depicts either the State A or the State B. The 'Underlying Opponent Process' (in the middle) depicts the processes initiated by the stimulation, i.e. *a* process and *b* process. In panel A, the *a* process is induced by the stimulation and has similar properties as the stimulation (e.g. aversive properties if US is a painful electric shock), the *b* process follows the *a* process and has opponent properties (i.e. appetitive). The *a* process is strong while the *b* process is relative mild. In panel B, the *a* process is relatively mild while the *b* process is strong after repeated stimulation. This is expected after repeated stimulation. In line with this, State A is now weak and attenuated whereas State B is powerful and enduring.

(from http://images.google.de/imgres?imgurl=http://nsw.royalsoc.org.au/journal_archive/images/walker/walker1.gif&imgrefurl=http://nsw.royalsoc.org.au/journal_archive/walker.html&usg=__zfUKjIZiTACe1KLnrzrRsJOnR9A=&h=716&w=1311&sz=14&hl=de&start=14&tbnid=iLmpY2zv5KionM:&tbnh=82&tbnw=150&prev=/images%3Fq%3Dsolomon%2Bopponent-process%26gbv%3D2%26hl%3Dde%26sa%3DG)

expression for a few minutes, they usually smile, chatter, gesticulate, are very socially active and appear to be elated. In a second example with an appetitive reinforcer, a couple has just begun sexual foreplay and it is quite pleasurable. Unfortunately, at that moment a telephone rings. One partner leaves and goes into another room to answer it and the other partner lies in bed. The abandoned partner experiences a quick decline of the pleasure, and then becomes tense and irritated (for further examples, see Solomon 1974, p. 121). Hence, these two

examples show how in daily duties unpleasant feelings are followed by pleasant feelings and vice versa. Presumably, such an after-effect plays also a role in associative learning. The affective habituation refers to the diminished affective reaction to US, when a US of medium intensity is repeated many times within relatively short periods of time. The first presentation of a US induces a stronger State A, the amplitude of which is decrease after several presentations; but if the peak of State A decreases, the peak of the State B increases showing an asymmetrical pattern (Figure 1.2.). Finally, the affective withdrawal refers to the change of the hedonic potency of a reinforcer after the repetition of such reinforcer. Thus, the new reinforcer, which occurs after the termination of the original reinforcer, has a hedonic quality opposite the original reinforcement onset and becomes even stronger through the affective habituation.

Consistent with the classical Pavlovian conditioning, organisms acquire motives by means of an association. Thus, the US has drive properties to motivate or to reinforce specific behaviors. The stimuli are no longer rendered neutral by conditioning derived motives as a consequence of associative processes. Furthermore, a typical motivational phenomenon is characterized by opponent processes which are also reflected in the mammalian brain (Leknes, & Tracey, 2008). In other words, a primary *a* process for a given hedonic state (i.e., the State A) is aroused by an aversive or an appetitive stimulus and such an *a* process is then followed by a second sluggish *b* process. The *b* process has an opposite hedonic sign to that of the state aroused by the input and gives a *State B.* Because of its sluggish latency and slow decay after the US has been terminated, the *b* process drags down the strength of the *a* process. However, the magnitude and the qualities of the *a* process and the *b* process are fed to a summator that computes $|a - b|$ for any moment. The summator determines whether the subject is in *State A* or *State B* as well as the intensity of those states. The state rule is simple: If $|a - b|$ shows $a > b$ then the organism is in *State A*; if $|a - b|$ shows $b > a$ then the organism is in *State B.* Furthermore, if being in *State A* is negatively reinforcing (i.e., aversive, undesirable) then being in *State B* will be positively reinforcing (i.e., pleasant, desirable) and vice versa. Importantly, the reinforcing properties of a given *b* process must be correlated with the magnitude of State A, the magnitude of a State A must be correlated with changes in the magnitude of State B and such changes are non-associative in nature, coming about only because of the repetition of a reinforcer. This means that the weaker the State A is the stronger the *b* process is and the other way around. Repetition of State B and a stimulus may then induce an association between them and therefore, if *b* process is derived from aversive *a* process, it might provide a relatively enduring source of positive hedonic reinforcement.

Though Rescorla described his theory very well and based its observations on daily life, he did not provide experimental evidence. However, during the last years the literature increasingly sustains this theory. In fact, cortical, neural correlates seem to operate in such an opposite fashion.

Curiously, anatomical and pharmacological evidences suggest similar mutual opponent activation in the dorsal raphe serotonin system, the ventral tegmental (VTA) and the substantia nigra dopamine system (Daw, Kakade, & Dayan, 2002). At the moment, serotonin (5-Hydroxytryptamine; 5-HT) is one of the less well-known main vertebrate neuromodulators, however, it has been suggested that 5-HT acts as an opponent partner with dopamine. The serotonin system consists of two nuclei, the dorsal and median raphe nuclei, with the dorsal raphe making connections to those areas also innervated by the dopaminergic system. Serotonin seems a critical part of the defensive system, which triggers fight or flight responses and is generally concerned with adaptive responses to aversive events. On the other hand, dopamine is assumed to promote appetitive behavior such as approach or reactions to rewarding stimuli (Schultz, Dayan, & Montague, 1997). Importantly, dopamine is involved in activating behaviors that serotonin inhibits and vice versa. Fletcher and Korth (1999) showed that agonizing serotonin opposes conditioned and unconditioned behaviors that are activated by dopamine; agonizing dopamine or antagonizing serotonin has the opposite effect. Bassareo and colleagues (Bassareo, Luca, & Chiara, 2002) provided further evidence using microdialysis. Thus, dopamine release in the nucleus accumbens shell is indeed inhibited by brief and relatively weak aversive stimuli.

The opponent-process theory may also explain the growing literature that shows an activation of those neural networks, which are normally involved in the processing of reward, induced by delivering aversive stimuli. Indeed, Semyour and colleagues (Seymour, O'Doherty, Koltzenburg, Wiech, Frackowiak, Friston, & Dolan, 2005) used a painful stimulus as the US consisting in the administration of capsaicin to the lateral aspect of the left leg. In a forward conditioning paradigm, a stimulus predicted the increase of a painful temperature (the aversive US consisted in a temperature increase of 5°C) and another stimulus signaled the temperature decrease (the relief US consisted in a temperature decrease of 5°C). What they found was a correlated activity in the ventral striatum with the prediction of relief. Thus, striatum activation responded specifically to the cue predicting the decrease of pain (i.e., relief). And they concluded that pain-relief and reward might share a common neural substrate in this region.

### *1.1.3. The Sometime Opponent Process Theory (Wagner, 1981)*

If Solomon's theory accounts for the affective processes underlying stimuli perception, it does not explain how the associations between events are formed. Thus, a further extension of Rescorla and Solomon's theories has been suggested by Wagner (1981) in the *sometime opponent process* (SOP) theory.

The SOP theory asserts that any stimulus excites a node that consists of a set of elements. Normally the elements are in an inactive state, but they may occasionally be in one of two states of activation, A1 and A2. The A1 state of activation can be likened to the stimulus being at the focus of attention, whereas the A2 state can be likened to the stimulus being at the margin of attention. The only route by which elements in a node may enter the A1 state is by presenting the stimulus itself. There are, however, two routes by which elements may enter the A2 state. One route is through decay of the A1 state. The other route depends upon previously formed associations. If the representation of CS and US are both in the A1 state, which will occur if the stimuli have just presented, excitatory conditioning will take place and representation of CS is associated with the representation of US. Subsequently the presentation of the CS will excite US elements directly to the A2 state. If one representation is in the A1 state, e.g. CS, and the other one is in the A2 state, e.g. aversive US, inhibitory conditioning will take place and the conditioned responses to CS have opponent properties, e.g. attenuation of fear responses; if both representations are in the A2 state no learning will occur. Hence, when the CS (e.g., a shape or a tone) is presented by itself and the representation of US (e.g., electric shock or food) is activated to the A2 state, then CS and US are not associated.

## 1.2. Affective Associative Learning

Emotions are hierarchically organized and the superordinate division is between positivity (pleasant state: Joy, love) and negativity (unpleasant state: Anger, sadness, fear). The organization of response systems in emotion is founded on two basic motive systems, appetitive and defensive (Lang, et al., 1998) and emotions are considered to be action dispositions because they drive a reaction. It is held that affects evolved from reflexive, overt reactions to appetitive or aversive stimulation that served immediate survival function (e.g., nurturance, sexual approach, fight, flight). The two systems operate in an opponent way and can vary their arousal level. That is, arousal represents the intensity of activation (metabolic and neural) of either the appetitive or the defensive system. When the systems are activated enough, the behavioral strategy (either approach or avoidance) is determined by which system

is most activated, whilst the other system is inhibited. The valence of the systems refers to the quality of the responses. Thus, when the stimulation is appetitive, e.g. reward presentation, then the behavioral strategy would be approach, therefore indicating positive valence. When the stimulation is aversive, e.g. threat presentation, then the behavioral strategies would be flight or fight indicating negative valence. As I already said, a neutral stimulus in the Pavlovian conditioning paradigm comes to acquire new "emotional" properties by simple pairing with an affective unconditioned event. This neutral stimulus will therefore acquire aversive properties if associated with an aversive US, whereas it will acquire appetitive properties if the US is appetitive. Consequently, the organism reacts to the CS with approach or avoidance according to the acquired stimulus properties (i.e., valence). Hence consistently with the valence of the US, the associative learning is distinguished between aversive or fear conditioning (with aversive US) and appetitive conditioning (with appetitive US).

### *1.2.1. Fear Conditioning*

Fear can be one of the most potent emotional experiences of an organism's life. The strength of this subjective experience may be because fear serves a function that is critical to the survival of the organism allowing an animal to be biologically prepared for danger-relevant cues (Öhman, 2005). *Fear conditioning* in the form of Pavlovian conditioning is the most used paradigm in the psychological field because it seems to be an excellent model for unraveling the processes and mechanisms underlying learning and memory. There are some major reasons to investigate the neural basis of fear. First, fear-modulate behavior may be used as a model to understand how emotions influence behavior. Second, fearful experiences are rapidly learned about and long remembered. Third, disturbances of fear conditioning may contribute to anxiety disorders in humans such as phobia, panic disorders and posttraumatic stress disorder (PTSD) (Bouton, et al., 2001; Himadi,1980) and also provide a framework to study the development of aversive expectation and the interplay between cognitive and emotional processes during learned fear (Grillon, 2002). Once conditioning has occurred, the CS elicits a constellation of responses that have been designed through evolution to help the organism to adapt itself better to the environment. When, for example, the imminence of a predator is low, the animal goes about its daily business; but when the predator is nearby a different set of behaviors becomes functional. At this point, the animal might freeze, the heart rate might slow down and respiration becomes shallower. Numerous studies with both animals and humans showed that after fear conditioning the subject increases fear responses that is startle reflex, amygdala activation, avoidance responses in the presence of the stimulus

associated with an aversive event (Bechara, Tranel, Damasio, Adolphs, Rockland, & Damasio, 1995; Büchel, Morris, Dolan, & Friston, 1998; Cheng, Knight, Smith, Stein, & Helmstetter, 2003; for a recent review see Delgado, Olsson, & Phelps, 2006; Grillon, Baas, Cornwell, & Johnson, 2006; Grillon, Morgan, Davis, & Southwick, 1998; LaBar, Gatenby, Gore, LeDoux, & Phelps, 1998; Lipp, Sheridan, & Siddle, 1994; Lissek, Powers, McClure, Phelps, Woldehawariat, Grillon, & Pine, 2005; Menon, Jensen, Vitcu, Graff-Guerrero, Crawley, Smith, & Kapur, 2007; Moratti, Keil, & Miller, 2006; Phelps, O'Connor, Gatenby, Gore, Grillon, & Davis, 2001; Weike, Schupp, & Hamm, 2007). One of the first pieces of evidence in humans of the role of the amygdala in fear learning was reported by Bechara and colleagues (1995). The authors applied discriminative fear conditioning to either healthy subjects or to a patient with bilateral damage to the amygdala. A monochrome slide worked as CS and predicted the aversive US, i.e. a startling loud sound, whereas another slide was not associated with the US. The authors found that the amygdala has a crucial role in fear conditioning. In fact, healthy participants responded fearfully to the CS, i.e. increase of the skin conductance response (SCR) magnitude; whereas the patient with amygdala lesion did not acquired conditioned autonomic responses to CS. Interestingly, this patient was aware of the association between CS and US because the patient reported it correctly. Therefore, to learn how to predict an aversive US and to react appropriately, the amygdala is fundamental, but it is not necessary for having the explicit knowledge of the contingency between events. Supporting the role of amygdala in fear conditioning, both Büchel et al. (1998) and LaBar et al. (1998) found a stronger activation in the amygdala in the presence of CS during fear conditioning. However, amygdala activation was strongly found during the first trials of both conditioning and extinction. This result corroborates animal evidences, in which amygdala has been shown to change its responses quickly during learning. In fact, amygdala activity strongly increases in the first trials to decrease afterwards and this might represent the plastic change necessary for the acquisition of the conditioned response. Other studies did not take into account cortical activation as a conditioned response, but peripheral physiological responses like defensive startle response (Lipp, et al., 1994; Grillon, & Ameli, 1998b) or skin conductance (Hamm, & Vaitl, 1996) or behavioral reactions (Grillon, et al., 2006). The startle reflex is a fast muscle twitch evoked by an intense and sudden stimulus e.g. white sound and is an index of the fear state. The degree of startle reflex covaries with the activation of the defensive (startle amplitude is potentiated indicating fear or implicit negative valence) and appetitive (startle amplitude is attenuated indicating pleasure or implicit positive valence) motivational systems. Skin conductance response (SCL) is also used as a measure of fear, but

in this case it covaries with the arousal level of the stimuli and is an index of the orienting response associated with the activation of the sympathetic system. Heart rate (HR) response is determinate by the activation of the sympathetic and parasympathetic system. HR exhibits a triphasic pattern during picture viewing, with an initial deceleration followed by acceleration and subsequently a secondary deceleration. Generally, the acceleratory component reflects a motor preparation that has only been found during acquisition. Especially fear potentiated startle reflex has proven to be a useful paradigm because it has been observed in animals (Davis, Falls, Campeau, & Kim, 1993; Koch, 1999) and humans (Grillon, et al., 1998a). In Davis study (1993), the animals are trained to associate a neutral stimulus, e.g. a light, with an aversive stimulus, e.g. footshock. After few pairings, the CS induced a state of fear that was measured by the potentiation of the startle amplitude. Grillon and colleagues (1998a) highlighted for example that defensive responses were facilitated by a verbal threat and darkness. Thus, the presence of CS produces a general increase in a subject's aversive state during aversive conditioning. Participants indeed responded to CS with enhanced startle amplitude when CS signals an electric shock or when participants are in threatening situations like in a dark room. In a later study using virtual reality, Grillon et al. (2006) replicated these findings using a virtual reality. After fear conditioning, participants were asked to move through the virtual rooms using a joystick. Participants showed increased startle in the presence of the aversive CS compared to the cue not associated with the shock and strongly preferred the no-shock context showing behavioral avoidance of the shock-context Similarly, Lipp and colleagues (1994) demonstrated that only when US is really aversive does human fear conditioning occur and the fear responses to CS are enhanced. When however the US is no-aversive, fear responses are not modulated by the presence of CS. The crucial role of predicting threats for survival has also been highlighted in a really interesting study by Bradley and colleagues (Bradley, Moulder, & Lang, 2005.). The authors conditioned affective pictures (either positive or negative IAPS) with a light painful shock (the aversive US). Independently from the intrinsic valence of the pictures, the fear responses were potentiated pictures signaling the shock. Hence, affective stimuli lost their intrinsic modulation of the startle response and just reflected the activation of the defensive system because the anticipation of a threat is more important and effective for survival. However, the common affective modulation of startle responses (i.e., potentiation by negative pictures and attenuation by positive pictures) were found when the affective pictures did not signal the shock, again supporting the importance of threat prediction.

### *1.2.2. Appetitive Conditioning*

A neutral stimulus may also acquire new motivational significance through its association with an appetitive US, e.g. food or money. In this case the properties acquired through learning are rewarding and positive, consequently the CRs to CS are characterized by approach or liking. This kind of classical conditioning is called *appetitive conditioning*. Although the conditioning of appetitive stimuli has the same evolutionary significance as the conditioning of aversive stimuli, appetitive conditioning has been studied in humans less than the fear conditioning. A possible explanation for this gap may be the difficulty in finding a suitable appetitive stimulus which can elicit a physiological activation similar to the one elicited by the aversive stimuli. Hermann and colleagues (Hermann, Ziegler, Birbaumer, & Flor, 2000) found dissociation between aversive and appetitive conditioning, but the CR to the aversive CS were stronger and more easily detectable than the CR to the appetitive CS were anyway. Indeed, the rewarding properties of a stimulus are easily influenced by the context or by the needs of the organisms (Small, Zatorre, Dagher, Evans, & Jones-Gotman, 2001).

But what is a reward exactly? A reward is defined as any object or event that generates approach behaviors and is an outcome of decision making (Schultz, 2007). Hence, rewards are not defined by the physics and chemistry of their inputs, but by the behavioral reactions they induce that are subjective feelings of pleasure, exploratory or approach behaviors and frequency, intensity increase of behaviors that lead to rewards. Comparing aversive with appetitive conditioning, Gottfried and colleagues (Gottfried, O´Doherty, & Dolan, 2002) identified a temporal and regional dissociation between these two kinds of associative learning. Thus, three neutral faces (CSs) were repetitively paired with either pleasant or neutral or unpleasant odors (USs), a fourth neutral face was never paired to an odor. The regional and temporal regions for appetitive and aversive conditioning were found within the amygdala, the nucleus accumbens (NAcc) and the orbitofrontal cortex (OFC). Thus, nucleus accumbens activation was specifically related to the appetitive CS, whereas the amygdala was specifically related to the aversive CS. The orbitofrontal cortex, on the other hand, was implicated in both kinds of learning. This finding provides additional support that the OFC is specifically implicated in coding and extracting the affective information of a CS to prepare the organism for subsequent decision making (Cox, Andrade, & Johnsrude, 2005). In another study, O'Doherty and colleagues (O´Doherty, Buchanan, Seymour, & Dolan, 2006) observed strong activation in the ventral striatum and the midbrain cortical related to participant's preference for rewarding stimuli, i.e. appetitive flavors. In line with these results, human cortical structures like nucleus accumbens, striatum, OFC, thalamus and cingulate cortex were

strongly activated in reward processing after appetitive conditioning (Kirsch, Schienle, Stark, Sammer, Blecker, Walter, Ott, Burkart, & Vaitl, 2003). And such cortical areas are the domain of dopaminergic projections confirming the importance of the dopaminergic system in reward processing as animal evidences showed (Koch, Schmid, & Schnitzler, 1996; Parkinson, Olmstead, Burns, Robbins, & Everitt, 1999; Schultz, 2000). One of the first studies showing the role of the nucleus accumbens in appetitive conditioning was conducted by Koch and colleagues (1996). They lesioned either the amygdala or the nucleus accumbens in rats and looked at the startle response after appetitive conditioning. Normally, startle amplitude is attenuated after appetitive conditioning, but when the nucleus accumbens had lesioned, there was no inhibition of fear responses after appetitive conditioning. This result highlighted the crucial role of NAcc in mediating positive affects and inhibiting aversive ones. Parkinson and colleagues (1999) could even differentiate the role of the core and the shell of the NAcc in reward processing. Thus, the shell seems to be fundamental in stimulating motor CR to appetitive stimuli, whereas the core seems to be implicated in mechanisms underlying the expression of the CS-US association.

Interestingly, an overlap has been found of those cortical networks involved in reward processing and those involved in relief from pain that is in the termination of an aversive event (for a review see Leknes, & Tracey, 2008; Rogan, Leon, Perez, & Kandel, 2005; Seymour, et al., 2005). Such evidence suggests that the relief from an aversive/painful event might be processed as a rewarding event. Comparing the neural correlates of a safety signal (i.e., the CS predicting the absence of the aversive US) and a danger signal (i.e., the CS predicting the coming of the aversive US), Rogan and colleagues (2005) found reduction of startle responses in mice in the presence of the safety. Moreover, the behavioral exploration of a novel environment was increased in the presence of the safety CS indicating reduction of fear and anxiety responses. In humans, Seymour and colleagues (2005) found that the ventral striatum was strongly activated by the presence of a visual cue signaling the decrease of a painful heat. The authors concluded that a mesolimbic region rich of dopaminergic projections could be the cortical region which conveys an appetitive relief-related prediction error. This evidence however seems to contrast with the results of experiment by Bradley and colleagues (2005) in which the prediction of a safety period did not modulate the startle response as strongly as the prediction of a threat did. However, we should consider that the stimuli she used (i.e., IAPS) had a strong intrinsic valence that a “normal” neutral stimulus in classical conditioning does not have. Moreover, prediction of the absence of an aversive event may not have rewarding properties as strong as food or money.

### *1.2.3. Event Timing*

A final and integral part for this analysis on the associative learning is the role of the time and in particular of the *timing*. Timing refers to the temporal relationship between two stimuli. Spatial and temporal contiguity was one of the oldest principles in classical conditioning to form associations between events. Actually, a large body of data has demonstrated that simple temporal contiguity is not a sufficient condition for classical conditioning (for a review see Cooper, 1991). In fact, temporal arrangements between events may modulate the acquisition of conditioned responses. For example, simply the application of a short temporal gap between US and CS implicates distinct processes and neural circuitries (Burman, & Gerwitz, 2004; Clark, & Squire, 1998; Knight, Waters, & Bandettini, 2009; Knight, Cheng, Smith, Stein, & Helmstetter, 2004; Manns,Clark, & Squire, 2000; Weike, et al., 2007). In other

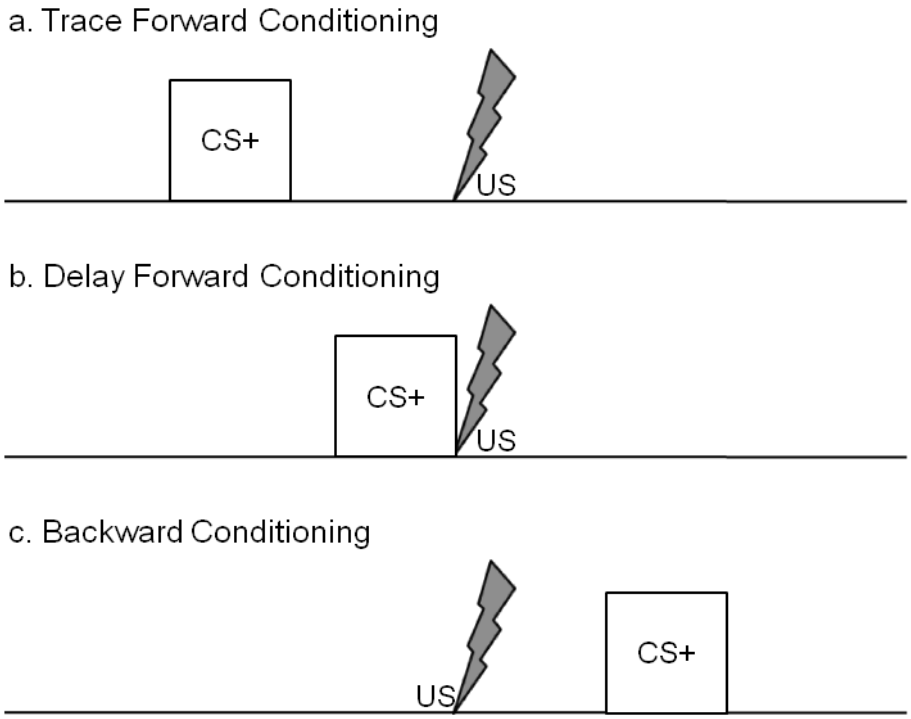



*Figure 1.3.* Schematic representation of temporal sequence between CS+ and US

The gray shock represents the aversive US, i.e. the painful electric shock. The square (CS+) represents the stimulus associated with the US during the acquisition phase. **a.** Trace forward conditioning indicates that between US onset and CS+ offset there is a temporal gap and US follows CS+. **b.** Delay forward conditioning indicates that US offset and CS+ offset coterminate. Again, US follows CS+. **c.** Backward conditioning indicates that US onset precedes CS+ onset.

words, reducing the temporal gap between CS and US induces a strengthening of the classical conditioning, whereas lengthening the gap's duration evidence of conditioning gradually deteriorates until there is no evidence. Trace conditioning is a procedure that involves a temporal gap between CS offset and US onset (Figure 1.3a) and has been found to be a hippocampus-dependent task (Hamm, & Weike, 2005). Delay conditioning is a procedure that does not involve temporal gaps between CS and US, i.e. they usually coterminate (Figure 1.3b) and it is hippocampus-independent. Evidence from animal and human research suggests that delay conditioning is a hippocampus-independent task (Clark, & Squire, 1998). Normally, conditioned responses are induced after trace conditioning, if participants develop awareness of the stimulus contingency, whereas participants react to the CS after delay conditioning if they have not explicit knowledge of the relationship between CS and US. Contingency awareness refers to the subject's knowledge of the association between CS and US and their ability to verbalize such association. Consistently, delay conditioning coincides with non-declarative memory, which may be expressed through habit, whereas trace conditioning coincides with declarative memory, which supports the capacity for conscious recollection of facts and events. Using a differential conditioning paradigm in which US was an aversive electric shock and CS neutral pictures, Weike and colleagues (2007) applied either a delay or a trace paradigm to two groups of participants. All participants showed potentiation of startle responses in the presence of the delay CS+. But, those participants who could not verbalize the contingency between CS and US did not show potentiation of startle response after trace conditioning and those who could verbalize the contingency shown increased startle responses in the presence of trace CS. These results support the crucial role of the hippocampus and declarative memory after trace learning. There is a large body of evidence on humans (Knight, et al., 2009; Knight, et al., 2004) supporting the neurophysiological findings provide by LeDoux (1995). LeDoux maintained that subcortical structures like the amygdala can be activated by direct projects from the thalamus and aversive learning might be a relatively low-level process that is not necessarily affected by a person's awareness of the CS-US contingency. Burman and colleagues (2004) used the fear-potentiated startle reflex in rats to compare the time course of fear expression after trace and delay conditioning. Their data demonstrated that fear responses in both trace conditioning and delay conditioning are related to the moment when the US occurred during the training. Thus, varying the temporal gaps between CS and aversive US as well as the CS duration, Burman and colleagues observed that the fear responses to CS in rats were maximally expressed

around the time of the scheduled occurrence of US. Therefore, animals encoded the time that elapsed between CS onset and US onset to react appropriately.

Thus far, I have referred to the association in which the US presentation follows the CS presentation either immediately or with a temporal gap. Such kinds of association are called *forward conditioning* (CS → US). In a traditional view, CS becomes a signal of the coming US because it precedes and predicts US and such a direction of the association seems to be the most functional for survival. However, many studies have illuminated the problem of the directionally of associations. Indeed, in his well-know study Ebbinghaus (1885) concluded that during the learning of a series of items, associations are made not only from a given stimulus that followed a second item (i.e., forward associations), but also from the given stimulus that preceded the second item (i.e., backward associations). *Backward conditioning* refers at the presentation of US preceding CS presentation (US → CS) (Figure 1.3c). Because of the fee evidences of backward conditioning, the content of learning after backward conditioning is poorly understood or mostly unanswered at this time.

Backward conditioning has empirically been found to affect behavioral reactions to the CS and such modulation entail opponent responses referring to the reactions to forward CS when it has been used with the same US (Arcediano, Escobar, & Miller, 2003; Chang, Stout, & Miller, 2004; Chang, Blaisdell, & Miller, 2003; Cole, & Miller, 1999; Hellstern, Malka, & Hammer, 1998; Salvy, Pierce, Heth, & Russell, 2004; Tanimoto, et al., 2004; Yarali, Ehser, Hapil, Huang, & Grerber, 2009). Tanimoto and colleagues (2004) found opponent conditioned reactions to a conditioned stimulus depending on the temporal sequence between CS and the aversive US. Thus, *drosophila melanogaster* avoided an odor associated with a painful electric shock when that odor had preceded a painful electric shock in a training phase (i.e., forward associations), but they approached an odor when it had followed the shock in a training phase (i.e., backward associations). Hellstern et al. (1998) demonstrated that backward pairings of CS and US induced conditioned inhibition (CI) in olfactory in the proboscis extension response (PER) in worker bees. The proboscis refers to the tubular feeding and sucking organ of a bee that allows the insects to eat. Extension of the proboscis may be interpreted as approach and inhibition of it as avoidance or an aversive reaction to a conditioned stimulus. The authors applied an odor as CS and a sucrose solution (reward) as US. After a few forward pairings, they observed that bees shown more approach response (i.e., extension of the proboscis) in the presence of such a conditioned odor. While, if the odor was backward associated with the sucrose, bees showed an opponent pattern of responses, thus inhibited the PER. Another example of backward associations was reported by Chang

and colleagues (2003). These authors conditioned rats backwardly using a footshock (duration 1 ms, intensity 1 mA) as US and a click train stimulus as CS (US → CS). In the test phase, Chang observed, on the one hand, that rats increased licking behavior in the presence of the backward CS when animals underwent just a few trials (i.e., 4). On the other hand licking was suppressed in the presence of backward CS when rats underwent many pairings (i.e., 96). These results indicate that the animals needed many US-CS pairing to learn that CS signals the absence of stimulation. In a really similar study, Cole & Miller (1999) applied a backward conditioning paradigm in which a sound (CS) was backward associated with a footshock (US). Again, suppression (inhibition) of licking was found only if animals underwent many backward pairings. Further evidence is brought by Salvy and colleagues (2004). The authors used the running wheel as US and drinking a sucrose solution as CS. Rats drunk less solution in a test phase when CS was repeatedly backward exposed to a running wheel (US → CS). This suppression of drinking is interpreted as result of conditioned taste aversion (CTA). Hence, if rats drank the sweet solution (CS) and then ran on the wheel (aversive US), they presented CTA (i.e., an avoidance response) afterwards despite the rewarding US. However, when rats first ran on the wheel and then drunk (CS → US), they presented a decrease of CTA afterwards (i.e., less avoidance responses). Nicely, Cunningham (Cunningham, Clemans, & Fidleer, 2002) found bivalence motivational (i.e., aversive and rewarding) effects in ethanol administration. The authors wanted to examine the ethanol-induced place conditioning in mice because there are no many evidences for this paradigm in mice. But surprisingly they found that conditioned responses were time-dependent. Thus, when the mice were placed in a chamber (CS) and then the ethanol (aversive US) was injected, animals showed conditioned place aversion (CPA). When, however, the mice received the ethanol and were then placed in the chamber, they showed conditioned place preference (CPP) afterwards.

This evidence from animals fits nicely with the Solomon's opponent-process theory (1980). Indeed, backward pairings between CS and US may induce association between the CS and the after-effect of US. Presumably, if CS occurs a few seconds after US cessation, then CS would coincide in time with the peak of the State B and therefore might acquire opponent properties than US. Interestingly, this idea that an aversive event may also have rewarding properties was proposed some decades ago by Himadi (1987), but since then no study has been done to test it. Indeed, Himadi suggested that not only the presentation of an aversive stimulus but also its termination has associative properties. Such termination, which may be called "relief" in the case where US is aversive, may then involve opponent responses compared to those normally elicited by the unconditioned event, e.g. inhibition of the fear

responses. Moreover, he proposed that the knowledge of such processes might explain the development of anxiety disorders like panic attacks. Thus, after a panic attack subjects feel relief and maybe they associated such feeling with a particular cue (e.g., doctor or nurse) then needing it to manage the "real world". However, this evidence in animals does not exclude the hypothesis that the absence of an aversive event has appetitive properties as Rescorla (1967), Seligman (1971) and Dickinson (1979) have hypothesized. In fact, a backward CS informs us that the US is already terminated, i.e. CS signals the absence of the US. Assuming that US is an aversive event, e.g. a painful electric shock, backward CS becomes an inhibitor of the defensive system (or as Dickinson suggested, an excitor of the appetitive system) and as a consequence appetitive responses to that CS are shown. Interestingly, pain and pleasure seem to share anatomical substrates which mainly involved the dopaminergic system but also regions like the nucleus accumbens and amygdala (Leknes, & Tracey, 2008). It might be due to this tight "neuronal" connection between the defensive and appetitive system that timing may turn an aversive event into reward.

In summary, both aversive and appetitive Pavlovian conditioning are useful and simple paradigms that explain how organisms make associations between events. Such formation underlies those mechanisms used by the organism to better adapt itself to the environment and assures the organism higher survival probability. Both US properties and event timing have a fundamental role in associative learning and this is of value to really simple organisms like fruit flies and complex organisms like humans alike. It remains however to characterize which mechanisms underlie backward associations and the acquired meaning of backward CS.

## 1.3. Neural Correlates of Associative Learning

### 1.3.1. *Neural Correlates of Fear Conditioning*

Current experiments have pointed out the neural circuitries of emotion processing extensively interacting with other brain regions underlying cognitive function. Based on animal evidence, LeDoux (1995) suggested that fear may be processed by two neural pathways that are the thalamo-amygdala pathway and the thalamo-cortico-amygdala pathway. The former is sufficient for the rapid triggering of emotion and for this reason is called the quick-and-dirty pathway or "low road". The latter involves cortical pathways before reaching the amygdala, is somewhat longer and slower, but the analysis of the emotional stimulus is more complete and thorough. This pathway is called the "high road". How it results is clear from the denomination of the pathways, the amygdala is a central region, crucial for both "quick-and-dirty" and "high-and-cognitive" processing of emotional (fear) inputs.

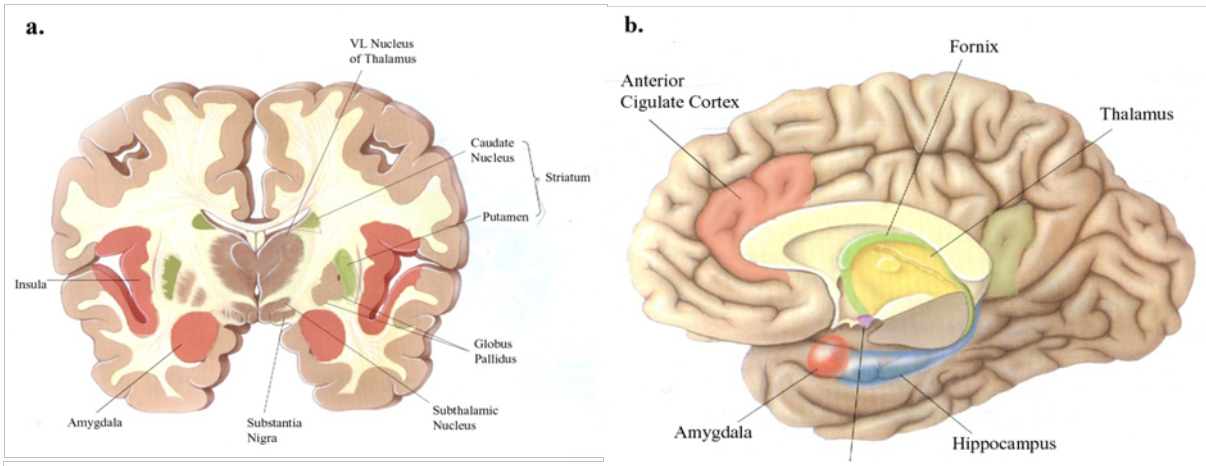



*Figure 1.4.* The regions of interest (ROI).

The red shading represent the cortical structures implicated in fear processing (e.g. amygdala, insula, anterior cingulate cortex). The green shading indicates the cortical structures implicated in reward processing (e.g. striatum, posterior cingulate cortex). **a.** Coronal view of a human brain (Bear, M.F., Connors, B.W., Paradiso, M.A. (1999) Il controllo cerebrale del movimento. In Bear, M.F., Connors, B.W., Paradiso, M.A. (Masson) *Neuroscienze. Esplorando il cervello.* (pp. 389). Milano Parigi Barcellona). **b.** Coronal view of a human brain (Bear, M.F., Connors, B.W., Paradiso, M.A. (1999) Il sistemi di memoria. In Bear, M.F., Connors, B.W., Paradiso, M.A. (Masson) *Neuroscienze. Esplorando il cervello.* (pp. 535). Milano Parigi Barcellona).

The amygdala (Figure 1.4a) plays the central role in the acquisition and expression of fear and fear conditioning. The notion that the amygdala might play a role in emotion first emerged when Kluver and Bucy (1937) demonstrated that medial temporal lobe lesions in monkeys resulted in a range of odd behaviors, including approaching normally feared objects, orally exploring objects and exhibiting unusual sexual behaviors. The amygdala is an almond-shape structure on the medial temporal lobe that sits adjacent and anterior to the hippocampus. It is the interplay between the sensory system that carries information about the CS+ and the US and the different motor and autonomic system that controls the conditioned reactions (Fendt, & Fanselow, 1999; LeDoux, 1995). In simple terms, sensory information from the cortex and the thalamus is received by the amygdala which then projects to hypothalamic and brain stem targets that mediate conditioned responses (Delgado, et al., 2006; Price, 2003). The lateral and basolateral nuclei (BLA) of the amygdala are the site of cortical and thalamic inputs and where the CS-US association is believed to take place (Kim, & Jung, 2006). In turn, these nuclei project to a central nucleus (CeA) which then projects in part to hypothalamic and brain stem target areas that directly mediate specific signs of fear and

anxiety (Davis, & Whalen, 2001). Hence on the one hand, the basolateral nucleus is critically involved in associative learning processes and such processes give the conditioned stimulus access to the motivational value of its associated unconditioned stimulus. On the other hand, the CeA is responsible for mediating expression of fear and anxiety (Davis, & Whalen, 2001; Delgado, et al., 2006). Other supports for the crucial role of the amygdala in fear conditioning were reported by human neuropsychological and brain imaging studies. Patients with amygdala lesions displayed a selective impairment in the recognition of fear face expression (Adolph, Tranel, Damasio, & Damasio, 1994) and deficits in fear conditioning (LaBar, LeDoux, Spencer, & Phelps, 1995) referred to normal subjects. Thus, after a fear conditioning procedure, healthy participants shown increased SCR in the presence of the conditioned stimulus associated with an aversive unconditioned stimulus. However, patients with unilateral (LaBar, et al., 1995) and bilateral lesions of the amygdala (Bechara, et al., 1995) failed to exhibit increases in SCR to the CS+ (as measure of the conditioned fear responses) despite this they could rightly report on the contingencies between the CS+ and the aversive US having thus explicit knowledge about the association.

Electrophysiological recordings of amygdaloid neuronal activity support and extend the role of the amygdala in encoding and storing fear associations. Likely, the mechanism underlying associative learning depends on the long-term potentiation (LTP) in the amygdaloid N-methyl-D-aspartate receptor (NMDA – Miserendino, Sananes, Melia, & Davis, 1990). The basis of most models of learning is provided by the activity-dependent modification of synapses. Thus, Hebbian plasticity, in the form of long-term potentiation (LTP) and long-term depression (LTD) of the synaptic membranes, provides the basis of most models of learning. Hebb originally wrote: "*The general idea is an old one, that any two cells or systems of cells that are repeatedly active at the same time will tend to become 'associated', so that activity in one facilitates activity in the other*." (Hebb 1949, p. 70). *Therefore,* "*When one cell repeatedly assists in firing another, the axon of the first cell develops synaptic knobs (or enlarges them if they already exist) in contact with the soma of the second cell.*" (Hebb 1949, p. 63). *However, recent experimental results suggest that the strength of the activity-dependent plasticity might depend on the spike-timing-dependent plasticity (STDP)*". That is, the temporal sequence of two inputs determines whether synapses are potentiated or depressed. It seems therefore that the modification of synapses depends on the interplay between the dynamics of NMDA receptor activation and the timing of action potentials back-propagating through the dendrites of the postsynaptic membrane. In other words, synaptic plasticity depends on the relative timing of the pre- and post-synaptic spikes (Abbott, &

Nelson, 2000). Considering fear conditioning, CS presentation might elicit US presentation because the synaptic spike by CS presentation was timed with the synaptic spike by US presentation during the training phase. Further discoveries showed similar plasticity in BLA during fear conditioning (Quirk, Garcia, & Gonzàlez-Lima, 1997). Hence, the idea is that synapses increase firing by CS+ presentation and decrease firing by CS- presentation and such modulation may involve synaptic plasticity mechanisms such as LTP and LTD especially in BLA (Davis, & Shi, 2000; Maren, 2001).

Thus far, the studies presented here point out the fundamental role of the amygdala in fear conditioning. However, other cortical areas like the insula, the cingulate cortex, in particular the anterior cingulate cortex, the motor cortex and the hippocampus play a role in fear conditioning as well.

The cingulate cortex (CC; Cingulum means belt in Latin – Figure 1.4b) is a part of the brain situated in the medial region of the cortex. CC is divided into the anterior cingulate cortex (ACC), medial cingulate cortex (MCC) and posterior cingulate cortex (PCC). Such division is due to their different functions and their morphological structure (Vogt, Nimchinsky, Vogt, & Hof, 1995). The ACC includes Brodmann areas 24, 25 and 32 and is involved in assessing the motivational content of internal and external stimuli and regulating the context dependent behavior (Büchel, et al., 1998; Mohr, Binkofski, Erdman, Büchel, & Helmchen, 2005; Ploghaus, Tracey, Gati, Clare, Menon, Matthews, & Rawlins, 1999). Regarding fear conditioning, ACC has been found to be particularly involved in orienting responses to a conditioned stimulus (Buchanan, & Powell, 1982) and in anticipation of an aversive event (Dunsmoor, Bandettini, & Knight, 2007). Furthermore, Milad and colleagues (Milad, Quirk, Pitman, Orr, Fischl, & Rauch, 2007) suggest that ACC might be especially involved in the fear expression since they found strong activation in the presence of CS+ referred to CS- and such an activation positively correlated with the SCR. ACC has also been implicated in contingency awareness (Tabbert, et al., 2006). In fact, only participants, who showed contingency awareness of CS-US association, showed significant ACC activation in the presence of CS+, but participants who did not show contingency awareness, did not. Hence, the ACC seems to be implicated in stimulus-reinforcement association and in the attribution of emotional significance to conditioned stimuli. Interestingly, Cardinal and colleagues (Cardinal, Parkinson, Hall, & Everitt, 2002) suggested that the ACC may also act to prevent erroneous generalization between CS. Although there are many aspects of human and rodent ACC function that are not yet reconciled.

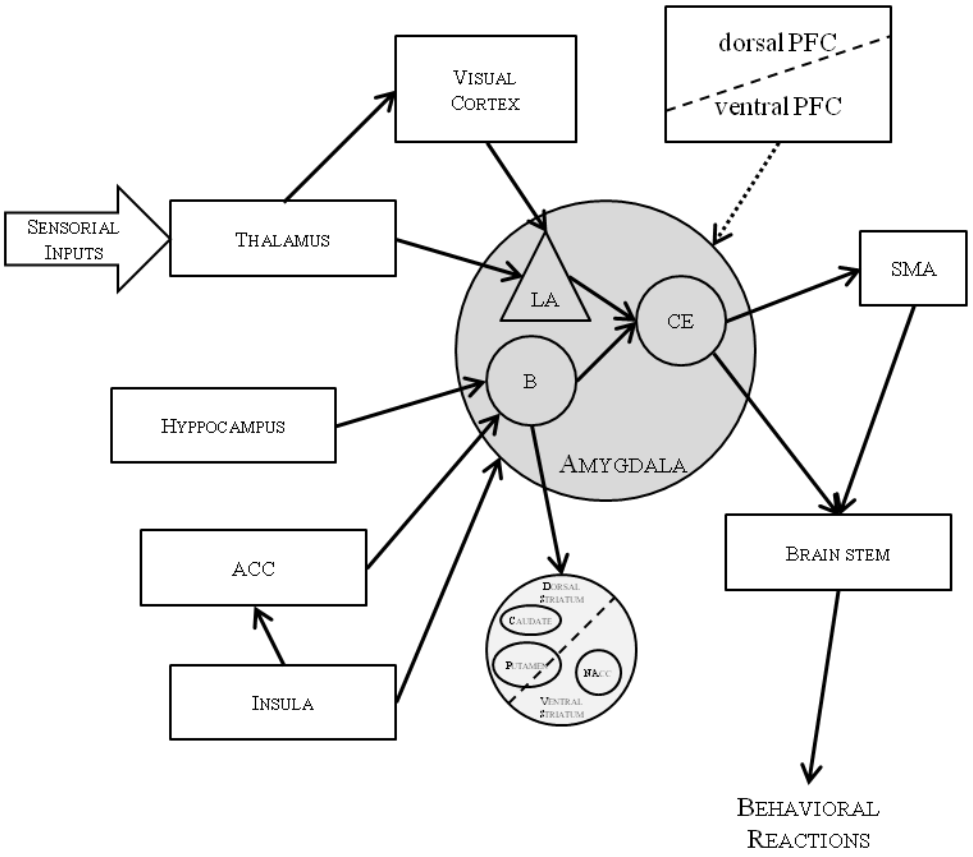



*Figure 1.5.* The projections of the defensive system.

The graphic summarizes the main neural areas implicated in fear expression and acquisition as well as the projections of the regions. A sensorial stimulation enters the central nervous system (CNS) through the thalamus. From where the information may be projected to the amygdala and undergo automatic processes (low road) or the visual cortex and undergo more cognitive processes (high road). The conditioned behavioral response is induced after an integration of the information from these two neural pathways. Moreover, amygdala activation may be modulated by the insula and ACC (which integrate automatic sensorial input with cognitive reflective inputs) and by the OFC (which has an inhibitory role on the amygdala activation).

The insula (insula is Latin for island – Figure 1.4a) is a structure of the human brain that lies deep to the brain's lateral surface, within the lateral sulcus separating the temporal and parietal lobes. The insula has been divided into various subregions. The more posterior regions of the insula receive inputs mainly from the thalamus and have been ascribed a role in somatosensory, vestibular and motor integration. The more anterior regions have been ascribed a role into emotional and motivational functions and have reciprocal connections to the "limbic" regions such as the ACC, the ventromedial prefrontal cortex (vmPFC), the

amygdala and the ventral striatum. Studies in the late 1980s indicated that the insula is linked to the asymbolia for pain in patients, characterized by the lack of an emotional response to painful stimuli (Berthier, Starkstein, & Leiguarda, 1988). And perhaps this is the first hint that the insula might be involved in the emotional and/or motivational experience. According to Damasio's model (Damasio, 1994) the insula provides an explicit (i.e., available to consciousness) representation of the bodily state that is elicited by emotionally stimuli. In other words, it is the place where sensorial inputs and cognitive processes are integrated. Several studies suggest a role of the anterior insula in fear conditioning (Büchel, et al., 1998) and pain (Ploghaus, et al., 1999). Interestingly, the insula has been found to be involved in the anticipation of pain and probably for this reason it is activated by a conditioned stimulus that had preceded a painful event (Porro, Baraldi, Paragnoni, Serafini, Facchini, Maieron, & Nichelli, 2002). Moreover, the insula responds to emotionally salient stimuli (Adolph, 2002) and is implicated in fear memory processing (Knight, et al., 2009). Finally because of their reach connections, ACC and the anterior insula probably provide one route through which the nociceptive input is integrated with memory to allow appropriate responses to stimuli that predict future adversities (Büchel, et al., 1998; Coghill, Sang, Maisog, & Iadarola, 1999).

The hippocampus (Figure 1.4b) is located inside the ventral medial temporal lobe and is associated with the dentate gyrus, with the parahippocampal gyrus and with the entorhinal cortex (the latter being the anterior portion of the parahippocampal gyrus, Brodmann's area 28). Its name probably derives from its curved shape reminiscent of a seahorse (hippos means horse in Greek and kampos means monster). The hippocampus has been subdivided into zones referred to as the CS (*cornus ammonis*) field based on differences in cellular morphology, connectivity and development (Gazzaniga, Ivry, & Mangun, 2002, p.84). The entorhinal cortex provides the main inputs to the dentate gyrus and the hippocampus and in turn it receives inputs from the CC. The hippocampus has been implicated in long term memory, spatial navigation and emotional processing (because of its interconnections with cingulate and mamillary bodies) (Kenser, & Hopkins, 2006). In fear conditioning, the hippocampus has been implicated in the formation of memory traces between the US and CS and in explicit knowledge of them (Squire, & Zola-Morgan, 1991). Comparing delay and trace conditioning, Weike and colleagues (2007) highlighted that declarative knowledge of the CS-US contingencies appears to have a differential impact on fear acquisition in delay conditioning compared to trace. Trace conditioning requires contingency awareness and is a hippocampus-dependent task. Delay conditioning does not depend on explicit awareness and therefore it is a hippocampus-independent task. Furthermore, patients with hippocampus

lesions whose amygdala are intact had a normal conditioned response as indicated by physiological measures, but no explicit knowledge of the relation between the CS+ and the aversive US (Bechara, et al., 1995). Animal findings also suggested a possible role of the hippocampus in memory processes related to temporal information (Burman, & Gewirtz, 2004). Indeed, rats showed maximal startle potentiation around the time of scheduled occurrence of an aversive US i.e. startle response was maximally potentiated just before US onset.

Finally I would briefly mention the role of the motor cortex in particular the supplementary motor cortex (SMA) and the premotor cortex (PCM). Since SMA is implicated in selecting movements reflecting internal goals and set learned pattern and PCM in selecting movements reflecting external stimulus information (Gazzaniga, et al., 2002, p. 497), they seem to be implicated in fear conditioning to generate a preparatory motor reaction to CS (Büchel, et al., 1998). PCM and SMA are a functional division of Brodmann's area 6 and these regions have direct projections from and to the spinal cord.

### 1.3.2. *Neural Correlates of Appetitive Conditioning*

So much for the neural correlates of fear conditioning, what about the neural correlates of appetitive conditioning? Previous evidence regarding appetitive conditioning suggests that the amygdala is not specifically involved in learning with appetitive US, since lesion of amygdala do not disrupt appetitive conditioning (Cahill, & McGaugh, 1990). But regions like the striatum especially the ventral striatum, the nucleus accumbens and the orbitofrontal cortex (OFC) are specifically implicated in the processing of rewarding events (Dayan, & Balleine, 2002; Jensen, Smith, Willeit, Crawley, Mikulis, Vitcu, & Kapur, 2007; Knutson, Adams, Fong, & Hommer, 2001a: O'Doherty, Dayan, Schultz, Deichmann, Friston, & Dolan, 2004; Pecina, 2008; Rolls 2000). The common characteristic of these regions is their dopaminergic projections. Dopamine (DA) is a neurotransmitter, a member of the catecholamine family, that is produced in the substatia nigra and in the ventral tegmental area (VTA) and it has long been identified with the processing of primary rewarding stimuli such as food, sex or secondary rewarding stimuli such as money (Wise, 2004). Schultz et al. (1997) discovered that dopamine neurons are strongly activated when animals receive a small quantity of fruit juice (i.e., reward) and such an activation is phasic, thus, several presentations of the rewarding stimulus are followed by a decrease in firing of the dopamine neurons. Interestingly, after repeated pairing of the cue followed by reward, dopamine neurons change their activation from just after time of reward delivery to the cue onset (Schultz, 2007)

indicating acquired appetitive properties by the cue. Hence, dopaminergic activity underlies associative processes between cue and reward. Other functions of DA neurons have been linked with incentive motivation, i.e. the drive-like effect of an otherwise neutral stimulus that acquires motivational importance through its association with a primary reward (Wise 2004). Indeed, DA neurons project their axons to brain structures involved in motivation and goal-directed behaviors such as the striatum, the nucleus accumbens and the frontal cortex. Notably, the neurophysiologic mechanisms proposed for appetitive learning are the LTP and the LTD exactly as for the aversive learning but in this case the dopaminergic neurons in striatum seem to be involved specifically (Di Filippo, Picconi, Tantucci, Ghiglieri, Bagetta, Sgobio, Tozzi, Parnetti, & Clabresi, 2009). In fact, lesions of the nigrostriatal dopamine system or application of D1 or D2 dopamine receptor antagonist or knockout of dopamine receptors impair LTD in striatum and the prefrontal cortex as well as LTP (Schultz, 2007). D1 and D2 receptors are also thought to make differential contributions to reward related learning. Thus, D1 receptors are important for learning new associations, D2 receptors enhance the influence of previously learned associations of appetitive behaviors (Aragona, Liu, Yu, Curtis, Detwiler, Insel, & Wang, 2006). This may explain why striatum has been found to be activated by both salience and rewarding stimuli. In fact, new evidence has implicated the ventral striatum not only in reward processing and prediction but also in fear processing (Delgado, Li, Schiller, & Phelphs, 2008) and in the development of contingency awareness in classical fear conditioning (Kluchen, Kagerer, Schweckendiek, Tabbert, Vaitl, & Stark, 2009a) supporting the role of striatum in the processing of novel and salient stimuli (Ungless, 2004).

Anatomical and pharmacological evidence suggests that the dorsal raphe serotonin system and the VTA-substantia nigra dopaminergic system may act as mutual opponents. Serotonin transporters (5-HTT) have been associated with anxiety-related traits and susceptibility for depression and the short-variant of the serotonin gene showed greater amygdala modulation during viewing of negative pictures (Canli, & Lesch, 2007; Hariri, Mattay, Tessitore, Kolachana, Fera, Goldman, Egan, & Weinberger, 2002). 5-HTT is released by the dorsal raphe nucleus and could act as a motivational opponent system to dopamine in conditioning tasks (Daw, et al., 2002). Similarly to the prevision of Solomon's model, 5-HTT may have an opponent and inhibitory effect on the DA. Thus, serotonin may be a critical part of the defensive system, which triggers fight/flight responses to aversive events, whereas dopamine might promote appetitive behavior such as approach. Daw and colleagues (2002) suggested that the balance between executing approach and withdrawal is determined by the balance

between dopamine and serotonin release in the ventral striatum. Interestingly, Rothman, Blough, and Baumann (2008) suggested a double of DA and 5-HTT in the cases of cocaine, alcohol addiction, obesity, attention-deficit disorders and depression.

The striatum is a subcortical part of the telecephalon and is the major input station of the basal ganglia. Anatomically, the striatum is the caudate nucleus and the putamen. Striatal activity seems to be involved in motor control and it also thought to be critical in controlling behavioral output (Schultz, Tremblay, & Hollerman, 2003). Many parts of the striatum are involved in reward processing and in various forms of learning and memory such as habit learning, goal-directed instrumental and reward-association learning and procedural and emotional learning (Schultz, et al., 2003; Yin, & Knowlton, 2006). The striatum is divided into ventral and dorsal striatum with distinct functions. The former is implicated in motivation and stimulus-reward learning, the latter in motor and cognitive control as well as in stimulus-response-reward learning (Cardinal, et al., 2002; O´Doherty, et al., 2004). A special region of interest included in the ventral striatum is the nucleus accumbens (NAcc). It is widely accepted that there are two major functional components of the NAcc, the core and the shell, which are characterized by different inputs and outputs. The core projects to conventional basal ganglia circuitry, whereas the shell projects to subcortical limbic structures. New findings indicate a functional dissociation between the core and the shell of NAcc (Corbit, Muir, & Balleine, 2001), that is the core is involved in evaluative processes via which the animals encode the incentive value of the outcome on the performance of goal-directed actions, instead the shell appears involved in mediating the excitatory effects of stimuli that anticipate reward on goal-directed performance. Moreover, Bassareo and colleagues (2002) highlighted that responsiveness in the NAcc shell is a function of motivational valence and novelty of a stimulus, whereas in the NAcc core it is an expression of generic motivational value. An interesting study by Koch and colleagues (1996) indicated that NAcc may to be involved in appetitive association. In fact, after having learned that a cue predicts a reward (i.e., sucrose liquid), rats normally showed attenuation of the fear responses such as startle reflex indicating a hedonic state, but such an attenuation was not shown when the NAcc was lesioned. Hence, NAcc mediates the hedonic state of an organism and reward prediction. Human evidence reported that activation in NAcc in anticipating rewards (i.e., money) but not punishments (Knutson, et al., 2001a).

The posterior cingulate cortex (PCC) includes 23 of Brodmann's areas and receives its dominant inputs from the hippocampus and the amygdala via the anterior thalamic and the laterodorsal nuclei (LD). In turn, it projects to motor areas, the cingulate areas and the caudate

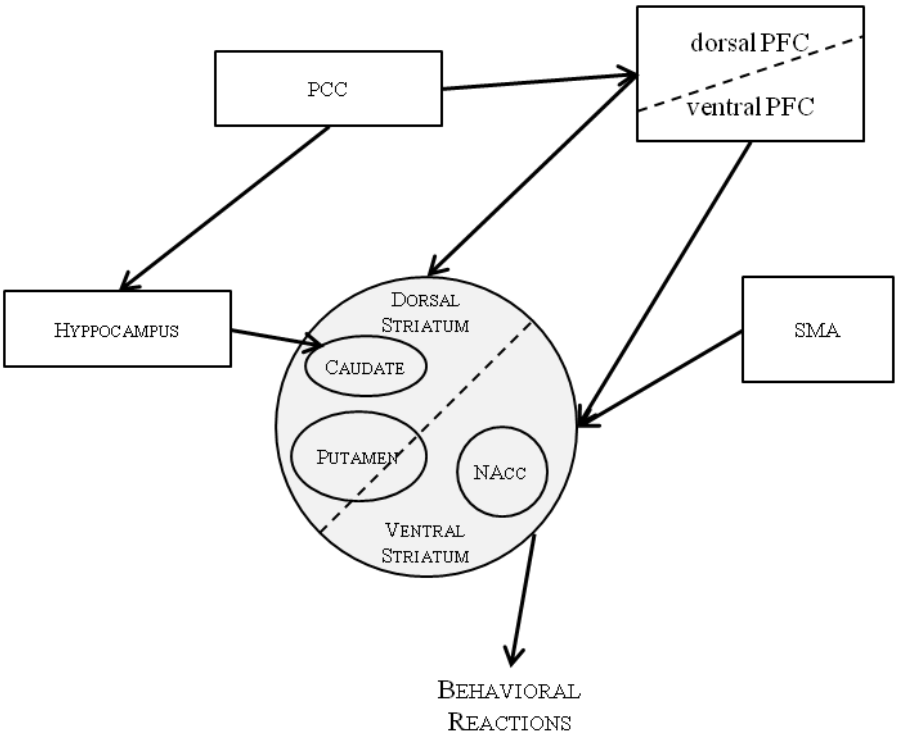



*Figure 1.6.* The projection of the appetitive system.

The graphic summarizes the main neural areas implicated in reward processing as well as the projections of the regions. Both OFC and the striatum are implicated in error prediction (i.e., the appraisal of the magnitude of a reward that is expected) and their projections presumes integration between a cognitive and an automatic pathways. These pathways may be considered as appetitive corresponding to the low and high roads in fear expression. Moreover and similarly to the amygdala, the striatum is the neural region in which appetitive associations take place and which seems to influence the memory processes in the hippocampus.

nucleus (Shibata, Kondo, & Naito, 2004). The posterior cingulate gyrus forms part of an attentional system with a role in the encoding of the associative significance of the stimuli while the anterior cingulate gyrus subserves executive functions (Fredrikson, Wik, Fischer, & Andersson, 1995) Pardo, Fox, & Raichle, 1991). Furthermore, PCC seems to have an interactive role in the evaluation of sensory inputs and movements, thereby providing maintenance of spatial orientation, memory, attention and evaluation of the significance of stimuli for the organism (Bromm, 2004; Fredrikson, et al., 1995). Hence, these neuroanatomical connections suggest that the PCC integrates sensory information and information from the limbic system to generate motor outputs. In an interesting study, McCoy and Platt (2005) reported that posterior cingulate neurons in the macaque cortex were

sensitive to reward size and its predictability suggesting a specific role in the processing of rewarding stimuli.

The last region (but not thereby the least important) of the appetitive network is the prefrontal cortex (PFC). PFC is the most anterior region of the frontal lobe and is involved in the higher aspects of motor control, planning and execution of behavior. The PFC is divided into three or more areas: the dorsolateral prefrontal cortex (dlPFC), which is found on the lateral surface of the frontal lobe anterior to the premotor regions and has been implicated in working memory functions; the orbitofrontal cortex (OFC) which is located in the frontal lobe's anterior-ventral surface and extends medially to limbic lobe structures with which it maintains interconnectivity (Gazzaniga, et al., 2002, pp.75); and the ventromedial PFC (vmPFC) which is located in the inferior region of the frontal lobe and has a role in reward-related decision making task (Gläscher, Hampton, & O'Doherty, 2009). The OFC represents stimulus reward value and subserves learning and relearning of association between arbitrary neutral stimuli and reward or punishment (Rolls, 2000). O'Doherty and colleagues (O'Doherty, Critchley, Deichmann, & Dolan, 2003) suggested that the OFC has a role in representing stimulus-reward values, signaling changes in reinforcement contingencies and consequently in behavioral control. Humans with OFC lesions are impaired on a number of tests of emotional reactivity to stimuli and make poor decisions (Bechara, Damasio, & Damasio, 2000). In a "gambling task", participants have to pick between two decks of cards, some decks pay out small rewards steadily for a net gain, with the occasional small loss, while other two decks pay out much larger losses (i.e., punishment) and occasionally rewards. Normal subjects learn to prefer picking from the safe decks and develop an autonomic response (SCR) that precedes their choice. The SCR is especially pronounced when participants are about to choose the risky decks. On the other hand, OFC-lesioned patients do not develop anticipatory SCRs and consistently perform poorly on the task.

In summary, two distinct neural networks for punishment and reward are presumably involved in fear and appetitive conditioning. The aversive neural system (specific for punishments) includes amygdala, insula and ACC, whereas the appetitive neural system (specific for rewards) includes the (ventral) striatum, PCC and OFC. After associative learning, a neutral stimulus associated with either an aversive or an appetitive US acquires new motivational properties that induce avoidance or approach depending on the valence of the US and whether the aversive neural circuitry or the appetitive neural circuitry are activated. Supposedly, the amygdala and striatum may be the "low road" for emotion processes and these regions then project to cortical regions like the ACC or OFC that will

elaborate the appropriated response to the stimulus integrating information from the memory, the expectancy and the goals of the organism. Of course, these aversive and appetitive systems are not separate, but they have interconnections that permit a prompt integration of the inputs.

### 1.3.3. *The Neural Projections of the Defensive and Appetitive System*

The cortical areas of the defensive and appetitive motivational system have a specific function as has been described in the previous sessions, but importantly they have rich interconnections. And it is the reciprocal influence that determines which kind of reaction is taken in response to CS since sensorial inputs are integrated with cognitive inputs.

In its elegant model of fear expression, LeDoux (1995) stated that there are two pathways for fear expression, a low road and high road. The former is a quick and automatic response to an external cue. The latter involves cognitive processes and is slower. These two pathways are also supported by neuroanatomical evidence. In fact, sensorial inputs project into the thalamus which projects either directly to the lateral nucleus of the amygdala (i.e., the low road) or to the visual cortex, which then projects to the amygdala (i.e., the high road) (see Figure 1.5.). Moreover, considering that fear memories are really strong, a possible explanation of this process is the tight interplay between the amygdala and hyppocampus (Phelps, 2006). Thus, the primary role of the amygdala is the acquisition and expression of fear (see 1.3. Neuronal Correlate of Fear Conditioning) and secondarily, the amygdala might influence the storage of memories in the hippocampus that is the amygdala may enhance the consolidation of the memories with emotional attribution. Both the insula and ACC project to the amygdala and this makes their role in mediating fear responses according to the sensorial and cognitive inputs plausible (Büchel, et al., 1998; Milad, et al., 2007).

The striatum receives synaptic inputs from cortical and subcortical afferents such as the motor cortex and PFC (see Figure 1.6) (Delgado, 2007). Moreover, just as the amygdala, the striatum seems to influence the consolidation process in the hippocampus (Johnson, Meer, & Redish, 2007). Thus, the information carried by the error prediction (which is “calculated” by the striatum) integrates the process of memory consolidation that mainly involves the hippocampus. Furthermore, both nucleus caudate and hippocampus are necessary for learning appropriate appetitive responses thought they seem to underlie two different systems for the acquisition of conditioned responses (Packard, & McGaugh, 1996).

### 1.4. Goal and Hypothesis

Based on all this evidence, I wondered if it would be possible to find opponent conditioned responses to a CS+ despite its association with an aversive US depending on the event timing. Thus, can event timing turn punishment into reward in humans as it has been found to do in fruit flies (Tanimoto, et al., 2004)? How is event timing reflected in cortical activation?

I hypothesized that the defensive motivational system and the appetitive motivational system (Lang, 1995) are activated differently depending on event timing. Thus, the defensive system would be activated after forward conditioning that is when US followed CS+ during the training phase (CS+ → US). And the appetitive system would be activated after backward conditioning that is when US preceded CS+ in the training phase (US → CS+) (Figure 1.7.).

Three studies were conducted to check these hypotheses. In one study, I have measured the startle responses to examine the acquired implicit valence of both CS+ after forward and backward conditioning. In a second study, I have investigated the cortical substrates of the two kind of learning using the fMRI. And finally, I have looked into the attentional processes underlying event learning since contingency awareness plays a crucial role in human classical conditioning.

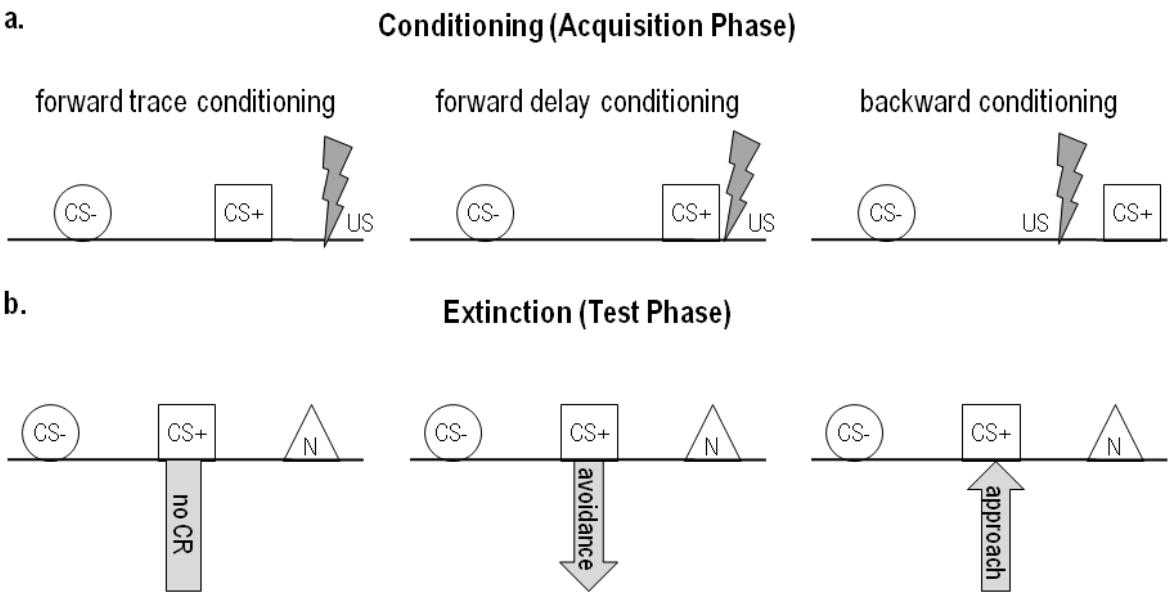



*Figure 1.7.* Graphical representation of the procedure and the hypothesis.

Classically, during a discriminative conditioning procedure, a stimulus (conditioned stimulus; CS+) is associated with a biologically significant event (unconditioned stimulus; US) and another stimulus is never associated with the event (CS-). **a**. During the acquisition phase, participants learn the association between CS+ the US. The temporal gap between CS onset and US onset is defined here as the interstimulus interval (ISI). Normally, US follows CS+ in a *forward conditioning* procedure; whereas US precedes CS+ in a *backward conditioning* procedure. The main difference between delay and trace forward conditioning is the temporal gap between US and CS+, i.e. trace conditioning procedure involves a temporal gap between CS+ offset and US onset, but delay conditioning does not. **b**. During the test phase, CS+ and CS- are presented again and in addition a neutral stimulus (N) is presented as control stimulus. No aversive US are delivered. Conditioned *avoidance* responses are expected after forward (delay) conditioning and conditioned *approach* responses after backward conditioning. No conditioned responses are expected when ISI was too long (forward trace conditioning).

## 2. The Startle Modulation Induced by Event Learning

The motivational priming hypothesis (Lang, et al., 1998) proposes that the affects are driven by two primary motivational systems: The appetitive system (consummatory, sexual and nurturant), prototypically expressed by behavioral approach, and the defensive system (protective and withdrawing), prototypically expressed by behavioral escape and avoidance. Such systems have opponent influence on each other, that is when one is active the other is inhibited. The startle response has been highlighted as convenient defensive reflex for testing this hypothesis. Startle response is a fast twitch of facial and body muscles evoked by sudden and intense tactile, visual or acoustic stimulus (Koch, 1999). The startle pattern in humans consists of eyelid-closure as well as an arrest of ongoing behavior. This response pattern is suggestive of a protective function against injury from predator and of the preparation of flight/fight responses. Moreover, startle has two main advantages (Davis, 1998). First, it is a cross-species defensive reflex; therefore, it allows translational studies between animals and humans. Second, its short latencies make it possible to determine the neural pathway that mediates such a reflex. Thus, startle reflex may be mediated by three central synapses: auditory nerves fibers to cochlear root neurons, cochlear root neurons axons to cells in the nucleus reticularis pontis caudalis (PnC) and pontis caudalis axons to motor neuron in the facial motor nucleus or spinal cord (Davis, 2006). Importantly, startle response has significantly greater amplitude when the aversive motivational system is active indicating a fear state, whereas it has an attenuated amplitude, if the activation of the appetitive system predominates indicating a state of pleasantness.

Fear-potentiated startle is defined as an increase in the amplitude of the startle reflex when it is elicited in the presence of a conditioned stimulus (CS+) that was previously associated with an aversive unconditioned stimulus (US) in contrast to when it elicited in the absence of this CS+. Fear-potentiated startle can be demonstrated in both animals (Davis, 1998; Davis, et al., 1993; Koch, 1999) and humans (Grillon, et al., 2006; Grillon, & Ameli 1998b; Lipp, et al., 1994 – Figure 2.1) and the amygdala seems to modulate such responses through its projection to the PnC. Evidence about the startle reflex such as a measurement of the defensive system were carried out by Grillon and colleagues (1998b). They used the startle reflex methodology to examine affective responses elicited by the anticipation of a threat. The authors applied a classical conditioning paradigm using an electric shock as aversive US and a light as CS+, they also used a further light (CS-) that was not associated with the shock. Furthermore, two additional conditions were used that is dark and light, during which participants received startle probes without administering electric shocks. Grillon and colleagues found that the

darkness facilitated the startle response and also that startle was facilitated by the light signaling the electric shock, i.e. the threat. Hence, startle response showed increased amplitude in threatening situation (i.e., the darkness and the aversive CS+) and this indicates that defensive responses are primed. Lipp and colleagues (1994) found similar results. They applied a paradigm that was similar to Rescorla's that is one group (conditioning group) received the CS+ associated systematically with the US (i.e., a painful electric shock) while another group (control group) received the same amount of CS+ and US but without any contingency between them. They found that startle amplitude was potentiated in the group that underwent the conditioning paradigm but not in the group that underwent the control paradigm. Hence, these results point out that fear conditioning engages processes that are not activated during non-aversive conditioning. In another interesting study, Grillon and colleagues (2006) highlighted the importance of the predictability of a threat and its utility in distinguishing between fear and anxiety. In a fear conditioning paradigm, they used a light (CS+) as predictor for a shock and a light as no specific predictor for the shock, but both consistently delivered in one room. In this case, the room would be associated with the shock becoming the CS+. Reputedly, such a context-CS+ elicits more anxiety, whereas the light-CS+ elicits fear; since in the former case the participants is not capable to predict the coming of the shock with certainty, whereas in the second case the participant can. Startle amplitude was higher in the presence of the threat-signaling stimulus (CS+) and also in the context associated with the shock without the specific cue. These findings support the idea that predictability is a key variable in the etiology and maintenance of anxiety (Barlow, 2000).

If the startle response is potentiated in a fearful/anxious context and by threatening cues indicating fear and avoidance tendencies, appetitive stimuli like sucrose can induce an attenuation of startle amplitude (Koch, et al., 1996; Lang, Bradley, & Cuthbert, 1990 Schneider, & Spanagel, 2008) and indicate approach tendencies. According to Lang et al. (1990), startle reflex indexes the strategic valence disposition of the organism. Thus, reflex amplitude will be enhanced linearly as the foreground stimuli vary from highly positive, appetitive content to highly negative, aversive content. In other words, startle amplitude is potentiated in the presence of aversive stimuli, but attenuated in the presence of appetitive stimuli indicating a hedonic state in this case. Koch and colleagues (1996) detected the role of the NAcc in the attenuation of the startle reflex. They conditioned 29 albino rats using a sucrose solution as US and a light as CS+. Some rats were lesioned in the NAcc and others in the amygdala. Interestingly, startle response attenuation was observed only in the presence of the CS+ associated with the appetitive US but not in the presence of the unconditioned reward

(the sucrose solution). Moreover, attenuation of startle response was disrupted in the group with lesions in the NAcc and not in the group with lesion in the amygdala. The authors concluded that such disruption of startle attenuation is consistent with the view that the release of dopamine in the NAcc is involved in the conditioned hedonic effect of reward.

Thus far it has been shown that changes in startle amplitude seem to reflect the disposition of the organism to react to a stimulus: dispositions to an aversive stimulus are characterized

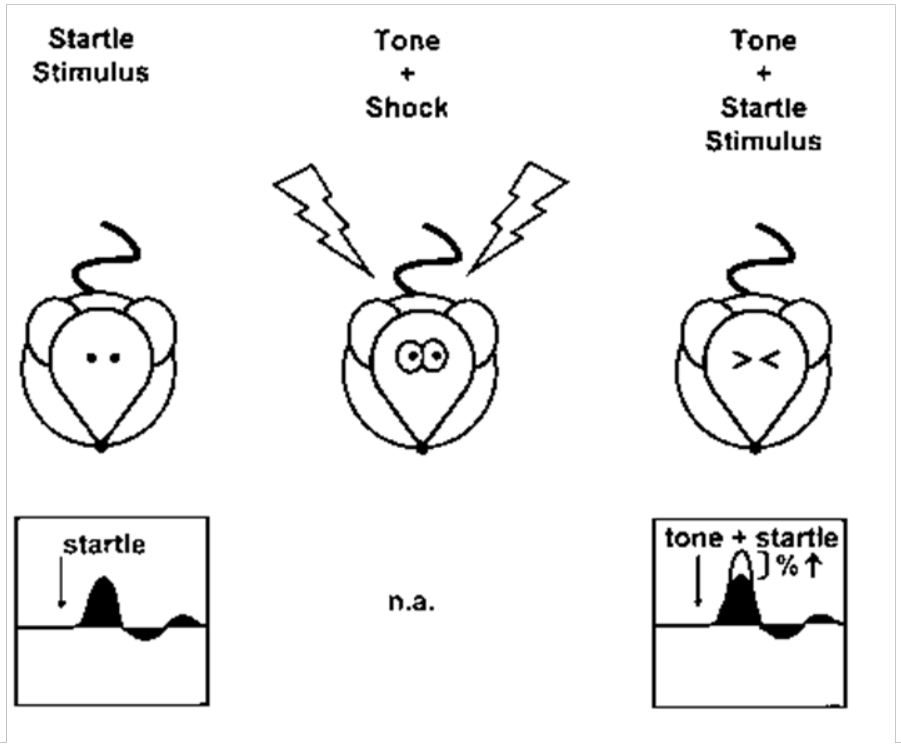



*Figure 2.1.* Fear-potentiated startle response.

Startle response is an ancestral defensive reflex, evoked by a loud and unexpected stimulus. **b**. During learning phase (i.e., the classical conditioning), an organism learns the association between an electric shock (aversive US) and an initial neutral tone. **c**. After learning, the tone (now called conditioned stimulus; CS) has acquired aversive properties and the animal reacts to the CS similarly as to the shock. Thus, startle amplitude is potentiated in the presence of the CS (from http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=frcogimp&part=ch12).

by avoidance, whereas dispositions to an appetitive stimulus by approach. Possibly, startle response is an implicit measure of the valence of a stimulus (Mucha, Pauli, & Weyers, 2006) showing the motivational orientation (avoidance vs. approach) of the reactions. Supporting this, potentiation of startle responses which indicates fear state and activation of the defensive system, seems to be modulated by projections from the amygdala to the PnC; whereas startle

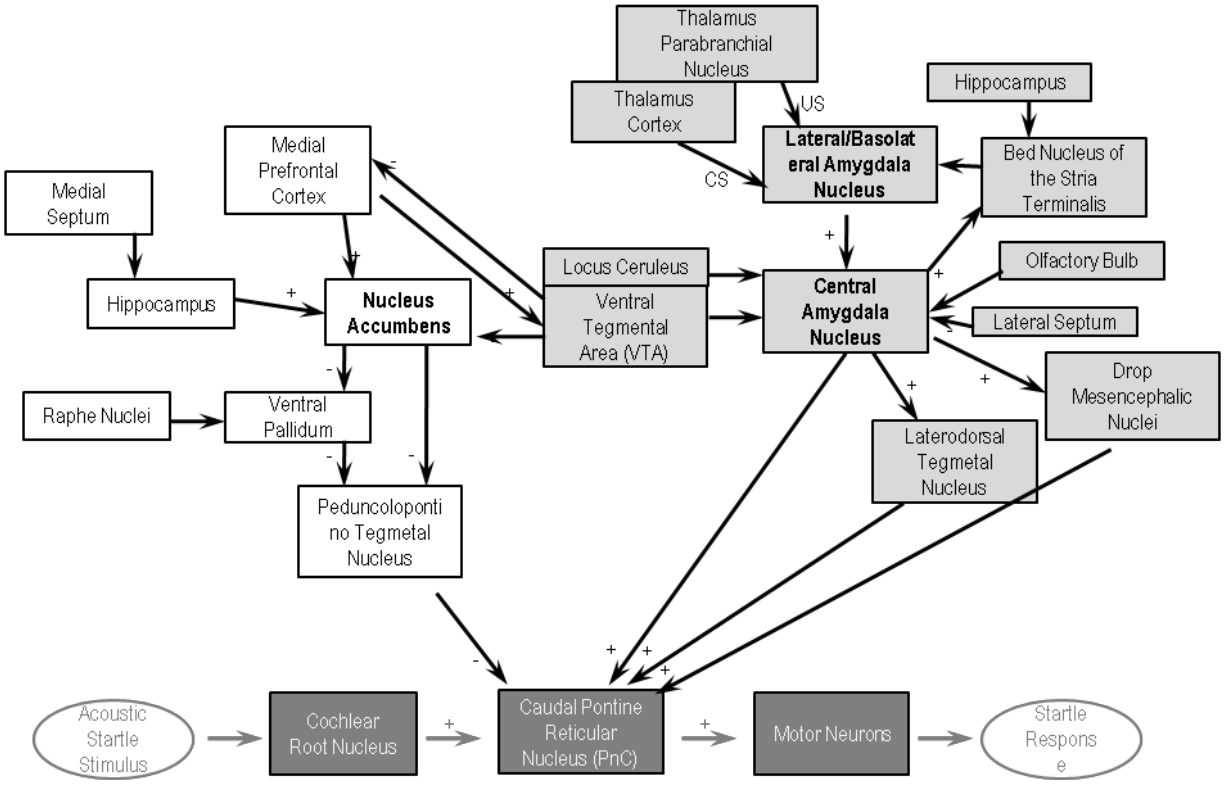



*Figure 2.2.* Sketch of the neurobiology of the startle response.

This graphic depicts a hypothetical circuit mediating potentiation and attenuation of startle responses according to Koch's (1999) review. The darkly shaded boxes symbolize brain areas that are involved in startle potentiation; whereas the lightly shaded boxes symbolize brain areas involved in startle attenuation. The algebraic symbol + indicates excitatory transmitter action; whereas the algebraic symbol – indicates inhibitory. DA is the abbreviation for dopamine, NA for noradrenalin, Ach for acetylcholine and GABA for γ-aminobutyric acid. CS and US indicate conditioned and unconditioned stimulus respectively.

attenuation, which indicates appetitive state and activation of the appetitive system, seems modulated by projections form the NAcc to PnC (Figure 2.2.).

Notably, dual-process theories (Bechara, 2005; Strack, & Deutsch, 2004) propose that human behavior is determined by the outputs of two interaction systems, an impulsive, implicit system working by associative principles, and a reflective, explicit system following decisions on the basis of knowledge about facts and values. Importantly, these systems can operate in a synergistic or antagonistic fashion to control the behavior. This reflective-impulsive model seems to share several aspects with Lang's (1995) motivational priming hypothesis. Thus, the impulsive system, which may be oriented toward avoidance or approach, seems to reflect the two motivational systems, i.e. appetitive and aversive.

Importantly, the reflective-impulsive model of social behavior also considers the reflective-cognitive process that characterizes humans. Hence, according to these observations, explicit and implicit valence of a conditioned stimulus may involve different processes. And startle response seems to perfectly fit as an implicit tool for testing the conditioned response after event learning; whereas subjective reports about the stimulus affective properties may fit well as an explicit tool. Furthermore, the fear conditioning paradigm provides a clear link to the animal literature as well as a framework in which to study the development of aversive expectation and the interplay between cognitive and emotional processes during learned fear.

As I mentioned in the introduction, there is a growing literature with animals (Cunningham, et al., 2002; Salvy, et al., 2004; Tanimoto, et al., 2004) showing how the sequence of US and CS+ (i.e., event timing) modulates the conditioned reactions to CS+. Thus, if CS+ has been preceding an aversive US, it then increases fear responses, whereas if CS+ has been following an aversive stimulus, it decreases fear responses. It therefore seems reasonable to expect that the temporal sequence of CS+ and US influences the affective responses to the conditioned stimulus in humans as well. Despite the numerous studies with animals, to best of my knowledge there is just one study (Mallan, Lipp, & Libera, 2008) that deepened the role of event timing in humans. A better understanding of event timing could be especially relevant to understand anxiety disorders (Grillon, 2002; Himadi, 1987) or drug addiction (Koob, & LeMoal, 2001; Weiss, 2005).

The aim of this study is to investigate the effects of timing on humans. To determine how event timing affects the response after event learning, I collected verbal reports as measure of the explicit valence (reflective system) and the startle reflex as measure of the implicit valence (impulsive system). I hypothesized that the CS+ valence would be rated more negative and arousing compared to a control stimulus after forward conditioning; whereas, after backward conditioning it would be rated more positive and less arousing compared to the control stimulus. Furthermore, I hypothesized that startle response in the presence of forward CS+ would be potentiated, but attenuated in the presence of backward CS+ both compared to the control stimulus. In order to test these hypothesis, I conducted two studies using a similar differential paradigm in which one stimulus was associated with a painful US and another stimulus was never associated with the US. In a between-subjects design, I compared the conditioned responses of participants who underwent either a forward conditioning or a backward conditioning.

## 2.1. Forward and Backward Delay Conditioning (Experiment 1)

In Experiment 1, I tested the opponent modulation of event timing on the startle responses after either forward or backward conditioning. Since there was no evidence using backward conditioning in humans, I applied a delay forward conditioning paradigm and its backward correspondent. In other words, the US was delivered either at the CS+ offset for the forward conditioning group or at the CS+ onset for the backward conditioning group.

### 2.1.1. Method

*2.1.1.1.Participants*

Thirty-seven university student volunteers (22 women, mean age = 21.89 years; SD = 4.1; range from 18 to 42 years old) participated in the study. They were free of neurological, psychiatric or chronic pain diseases. The sample was divided into the two experimental groups with 20 participants for the forward conditioning group and 17 participants for the backward conditioning group.

*2.1.1.2.Stimulus Material*

The *unconditioned stimulus* (US) consisted of a single unipolar electrical shock of 200 ms duration delivered via a surface bar electrode, which was attached to the left forearm (Neumann, & Waters, 2006). The bar electrode consisted of two durable gold-plated stainless steel disk electrodes with 9 mm diameter and 30 mm spacing. The stimuli were generated by a battery-driven constant-current stimulator (developed by the university of Konstanz), supplying a maximum of 140 V and a maximum of 10 mA. Before the experimental session, we assessed the pain threshold for each participant using two series of electric shock with ascending and descending intensity in steps of 0.5 mA (Reiff, Katkin, & Friedman, 1999). Participants evaluated the intensity of each electric shock on a visual scale presented on a computer screen and ranging from 0 (no pain at all) until 10 (unbearable pain). The mean value of the intensities rated as ‘just noticeable pain’ (i.e., 4) was defined as pain threshold.

Three simple geometrical shapes (a square, a circle and a triangle) served as visual *conditioned stimuli* (CS) in the differential conditioning paradigm. All stimuli were yellow, 12 cm in width and 12 cm in height (Lipp, et al., 1994). Visual stimuli were 8 s in duration and presented on a 19´´ computer screen with a black background. The screen was located 140 cm from the participant at eye level. Shapes were counterbalanced among the participants having the function of either CS+ (the shape associated with the US) or CS- (the shapes never associated with the US) or NEW (the control shape presented only during the test phase). The

temporal interval between CS+ onset and US onset is defined as the interstimulus interval (ISI) and it was 8 s for the forward group and 0 s for the backward group.

A burst of white noise of 105 dB with a duration of 50 ms served as a *blink-eliciting stimulus*. The acoustic stimuli were presented binaurally over headphones. Startle probes occurred 5-7 s after the shape onset. Eight additional startle probes were delivered during the interstimulus interval (ITI) in order to assure their unpredictably. The ITI is defined as the interval between the stimulus's offset and the stimulus's onset on a subsequent trial.

*2.1.1.3. Procedure*

After having signed the informed consent form (approved by the ethic committee of the 'Deutsche Gesellschaft für Psychologie', DGPs), the participants were seated in a comfortable chair in a sound-attenuated room next to the observation room. After electrode attachment, the pain threshold was assessed as described above. The participants were then told that a series of geometrical shapes would be presented and that they should keep these pictures in their visual focus. The participants were also told that the electric stimuli would be delivered without mentioning the contingency between CS+ and US and that in a successive phase a loud sound would be presented.

The experiment consisted of two main phases: The conditioning (i.e., the training phase) and the extinction (i.e., test phase). Before and after conditioning, participants were asked to rate the valence and the arousal of the square, the circle and the triangle using a visual analogical scale (VAS) from 1 until 9. 1 meant 'very unpleasant' and 'calm' and 9 meant 'very pleasant' and 'exciting' respectively. The participants saw the scale on a computer screen with a black background and had to push a number button on a keyboard corresponding to their rating. At the end of the rating, the conditioning began. Conditioning included 16 presentations of CS+, which was always reinforced with the painful electric shock, and 16 of CS-. The ITI varied from 20 s to 30 s. Before the extinction, nine startle stimuli were delivered every 7-15 s to decrease initial startle reactivity, such that both startle potentiation and startle attenuation could be detected easily. Extinction trials were identical for all participants, during which no electric shock was delivered. The three visual stimuli (CS+, CS- and NEW) were presented 16 times each. Eight startle-inducing noises were delivered during either CS+, CS- or NEW and 8 additional probes during the ITI. In all, 32 startle stimuli were delivered.

Immediately after the conditioning and the extinction, contingency awareness between CS+ and US was measured using a third VAS ranging from 0% to 100% (Knight, Nguyen, &

Bandettini, 2003). 0% indicated that the electric shock was never presented with that shape and 100% indicated that it was always associated.

*2.1.1.4. Physiological Recording and Data Reduction*

The eye-blink component of the startle reflex was measured through electromyography (EMG) of the left *orbicularis oculi* muscle (Figure 2.3.) with two 5 mm Ag/AgCl electrodes. One electrode was placed under the pupil of the left eye and the second one at approximately 1 cm laterally. Both the ground and reference electrodes were placed on the forehead. Before attaching the electrodes, the skin was cleaned with alcohol and slightly abraded to keep all electrode impedances below 5 kΩ. The raw signal was sampled at 400 Hz. Startle responses were registered continuously with a FirstAmp 16 using Vision Recorder V-Amp Edition Software (Version 1.03.0004; BrainProducts Inc.). EMG activity was filtered with 50 Hz notch filter to eliminate 50 Hz interference.

Offline analyses were conducted with the Brain Vision Analyser Software (Version 1.05; BrainProducts Inc.). Data were first filtered using a 28 Hz low cutoff filter and a 500 Hz high cutoff filter. A moving average of 50 ms was applied and then the myographic signal was rectified. The peak amplitude of the blink reflex was defined as the maximum of the integrated response curve in the 20 ms to 120 ms time window following probe onset relative to baseline (average baseline EMG level for the 50 ms immediately preceding stimulus onset – see Grillon, et al., 2006). Responses to startle probes were scored manually and trials with excessive baseline shifts or movement artefacts were excluded. Twelve participants were not included in the analysis. The raw data were then standardized using *z*-score conversions within subjects to normalize data and to reduce the influence of variability between subjects unrelated to psychological processes (see Blumenthal, Cuthbert, Filion, Hackley, Lipp, & Boxtel, 2005). *z*-scores are standard scores, which have a mean of zero and a standard deviation of one. The scores were averaged for each geometrical shape within the conditioning and within the extinction.

*2.1.1.5. Data Analysis*

All analysis was done with between-subjects factor conditioning (forward conditioning, backward conditioning), whereas the within-subjects factors were specific and different for each dependent variables. Thus, blink amplitude was assessed with the only within-subjects factor stimulus (CS+, CS-, NEW). The subjective ratings for the valence and the arousal were separately compared with the within-subject factors stimulus (CS+, CS-, NEW) and time

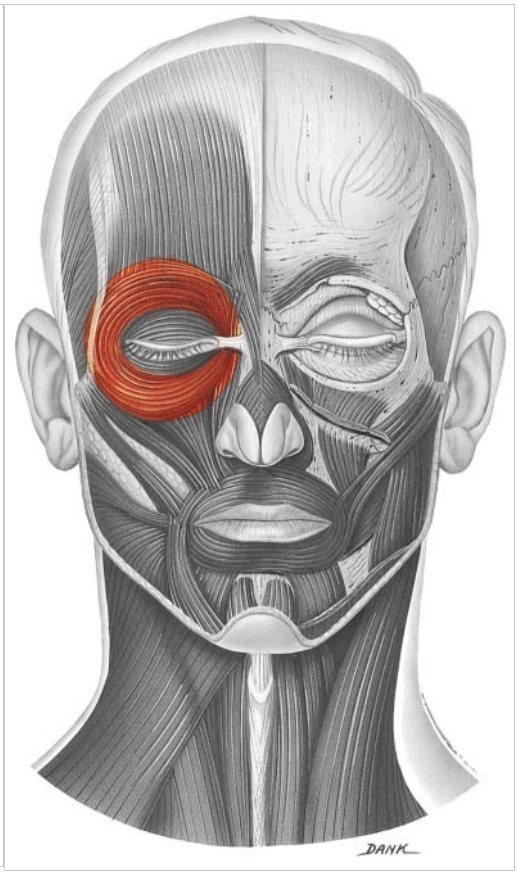
(before conditioning, after conditioning). The contingency awareness was analyzed with within-subjects factor stimulus (CS-, CS+) and time (after conditioning, after extinction). The α level was set at .05 for all statistical tests. Greenhouse-Geisser corrections (GG-ϵ) were used for the main effects and interactions involving factors with more than two levels.

All dates were analyzed using SPSS for Windows (Version 14.0.2, SPSS Inc.).

*Figure 2.3. Orbicularis Oculi* muscle.

Orbicularis Oculi is a striated sphincter muscle encircling the eyes in the face of humans. Such muscle allows the eyes to close. Electrodes are attached under the left eye to measure startle response, whilst isolated ground and reference electrodes were placed on the forehead.

from http://www.waukesha.uwc.edu/lib/reserves/pdf/zillgitt/zoo170/diagrams2/diagrams2.html



### 2.1.2. Results

*2.1.2.1. SubjectiveRratings*

Valence ratings varied as a function of the stimulus ($F(2, 70) = 6.86$, $p = .002$) and showed a significant Stimulus x Time interaction ($F(2, 70) = 5.41$, $p = .007$) (Figure 2.4a). Follow up

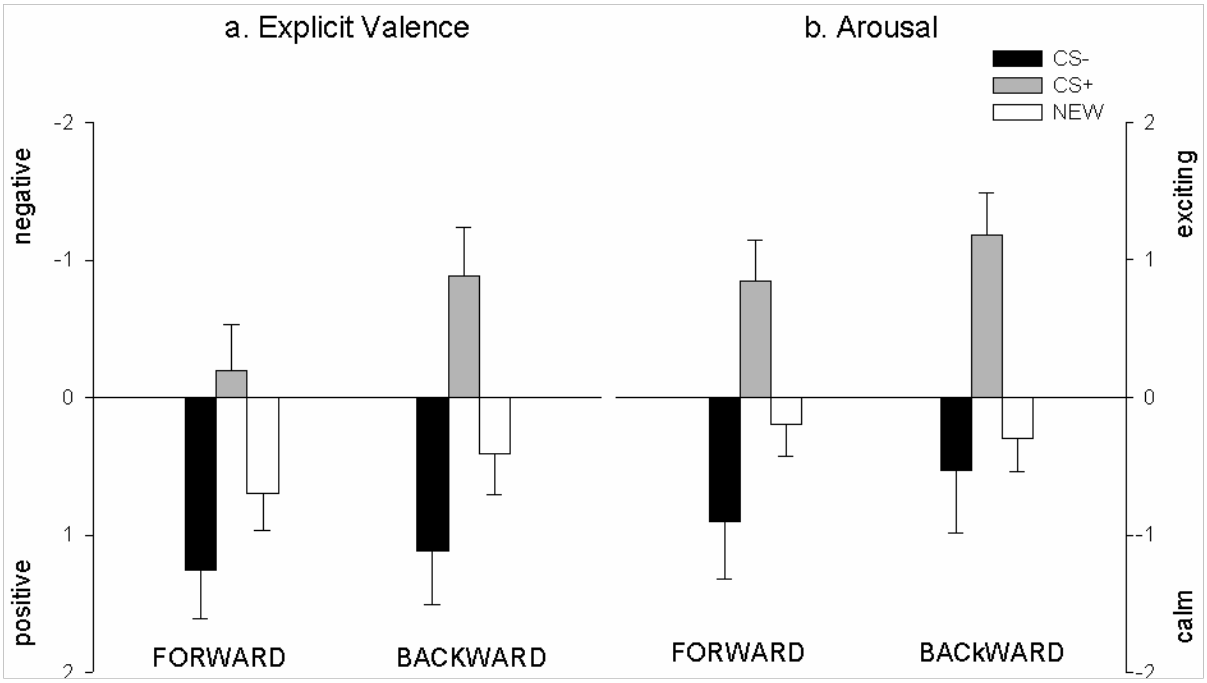



*Figure 2.4.* Explicit valence and arousal assessed with subjective ratings after forward and backward conditioning in Experiment 1.

Bars (with standard error) represent the ratings for the visual stimuli after forward conditioning and after backward conditioning. Black bars correspond to CS-; gray bars to CS+ and white bars to NEW stimulus. Participants rated valence and arousal of the visual stimuli using a visual analogical scale (VAS) from 1 (negative, calm) until 9 (positive, exciting) in which 5 meant a neutral rating (neither negative nor positive for the valence; neither calm nor exciting for the arousal). The values on the y-axis are differential values between the ratings and the neutral (i.e., 5) **a.** Ratings of the valence were consistently negative for CS+ and consistently positive for CS- after training phase indicating that stimuli reinforced during conditioning (CS+) were rated more negative and stimuli not reinforced during conditioning (CS-) were rated more positive compared to their initial ratings. **b.** Ratings of arousal were consistently higher for CS+ and lower for CS- after the training phase indicating that stimuli reinforced during conditioning (CS+) were rated as more arousing, whereas stimuli not reinforced during conditioning (CS-) were rated as less arousing compared to their initial ratings.

tests refer no differences among the three shapes before both forward and backward conditioning and the shapes were rated as 'emotionally' neutral. However, CS+ changed its valence significantly during the study. Thus, CS+ valence was significantly more negative compared to the NEW valence after both forward conditioning ($t(19) = -2.27$, $p = .035$) and backward conditioning ($t(16) = -2.42$, $p = .028$).

The overall ANOVA for the arousal ratings revealed significant main effects of stimulus ($F(2, 70) = 9.10$, $p = .001$, GG-$\epsilon = 0.78$) and a significant Stimulus x Time interaction ($F(2, 70) = 5.40$, $p = .010$, GG-$\epsilon = 0.85$) (Figure 2.4b). Follow up tests again indicated that arousal ratings did not differ among CS+, CS-, and the NEW before conditioning. However, CS+ acquired a significant higher arousal compared to the NEW stimulus after both forward ($t(19) = 3.2$, $p = .005$) and backward ($t(16) = 3.82$, $p = .001$) conditioning.

Forward and backward conditioning group did not differ concerning the valence ($F(2, 70) = 0.16$, $p = .857$) and the arousal ($F(2, 70) = 0.08$, $p = .899$, GG-$\epsilon = 0.85$) ratings.

*2.1.2.2. Startle Amplitude*

Startle data showed a significant main effects for stimulus ($F(2, 70) = 5.64$, $p = .005$) but not a significant Stimulus x Conditioning ($F(2, 70) = 0.49$, $p = .615$) interaction (Figure 2.5.).

Follow up tests indicated that the startle amplitude in response to CS+ was significantly higher compared to the startle amplitude in response to the NEW after both forward ($t(19) = 2.24$, $p = .037$) and backward ($t(16) = 2.19$, $p = .043$) conditioning. Startle amplitude in response to CS- did not significantly differ referred to the NEW stimulus (forward: $t(19) = 1.27$, $p = .221$; backward: $t(16) = -0.297$, $p = .770$).

*2.1.2.3. Contingency Awareness of CS-US Association*

The contingency data showed a significant main effect for the stimulus ($F(1, 35) = 80.84$, $p = .000$). Thus, US presentation was rated with higher probability in the presence of CS+ than CS- indicating that participants acquired contingency awareness for the association between US and CS+. Furthermore, the interaction Stimulus x Time had significant results ($F(1, 35) = 13.87$, $p = .001$). Follow up tests showed that the probability of US in the presence of CS+ was significantly higher compared to CS- after both forward ($t(19) = -5.88$, $p = .000$) and backward ($t(16) = -12.08$, $p = .000$) conditioning. However, participant contingency awareness decreased after the 'forward extinction' ($t(19) = 3.38$, $p = .003$) and almost also after 'backward extinction' ($t(16) = 1.91$, $p = .075$). Although, the awareness of the

contingency between CS+ and US was still marked (forward: $t(19) = -2.53$, $p = .020$; backward: $t(16) = -6.87$, $p = .000$).

A significant Stimulus x Conditioning ($F(1, 35) = 4.28$, $p = .046$) interaction was found indicating that contingency awareness after extinction was significantly different between

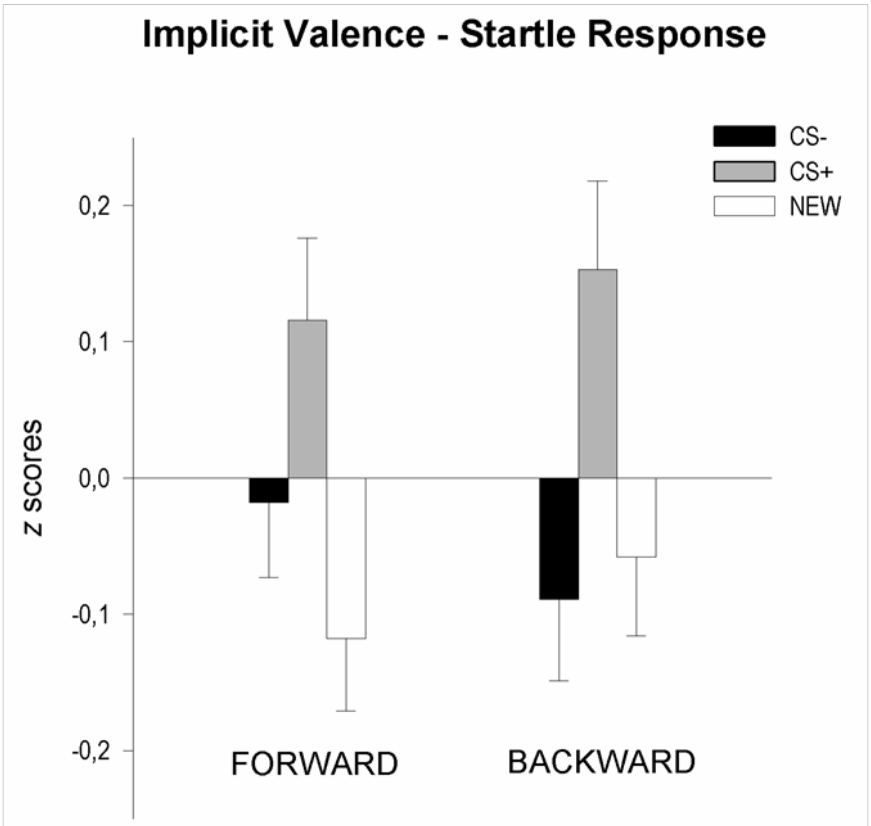



*Figure 2.5.* Implicit valence assessed with startle response after forward and backward conditioning in Experiment 1.

Bars (with standard error) represent the startle amplitude, black bars in the presence of the CS-; gray bars in the presence of the CS+ and white bars in the presence of the NEW. Startle responses were elicited only during the test phase after both forward (on the left side) and backward conditioning (on the right side). Positive values on the y-axis indicate startle response potentiation, negative values indicate startle response attenuation. During forward conditioning, the aversive unconditioned stimulus (US) followed visual conditioned stimulus offset (CS+); whereas during backward conditioning US preceded CS+ onset. The interstimulus interval (ISI), which is defined as the temporal interval between CS+ onset and US onset, was 8 s for forward conditioning and 0.2 s for backward conditioning. The picture shows a potentiation of startle amplitude in the presence of both forward and backward CS+ indicating implicit negative valence.

forward and backward conditioning groups ($t(35) = -2.67$, $p = .011$).

### 2.1.3. Discussion

The present study explored the modulation of the temporal sequence between CS+ and US on response acquisition. I hypothesised that depending on event timing the conditioned stimulus would assume opponent valence that is negative and positive valence despite its association with an aversive US.

The startle response is a fast defensive response that increases in amplitude when an organism is aversively motivated (i.e., when the defensive system is activated and the individual´s emotional state is affectively unpleasant) and decreases in amplitude when an organism is positively motivated (i.e., when the appetitive system is activated and the individual´s emotional state is affectively pleasant) (Grillon, et al., 2006; Grillon, & Baas, 2003; Koch 1999; Lang, Davis, & Öhman, 2000; Lipp, et al., 1994). When a neutral stimulus (e.g., a geometrical shape) reliably occurs contiguous with an aversive stimulus (e.g., an electric shock), then the feature of the defensive behavior comes to be evoked by that conditioned stimulus presented alone. In other words, the previously innocuous stimulus comes to activate a pattern of emotional responses as well as the unconditioned stimulus does. Exactly like the results of this study show. Thus, forward CS+ acquired the ability to predict the occurrences of the aversive US then operating as a danger signal and increasing fear responses. However, contrary to my initial hypothesis, backward CS+ increases fear responses as the forward CS+ did. Rather, backward CS+ does not enable participants to anticipate the electric shock as the forward CS+ because it follows the shock. Therefore, the conditioned responses to backward trained CS+ should undergo different processes.

It has been suggested (Chang, et al., 2004; Cole, & Miller, 1999; Cole, Barnet, & Miller, 1995; Heth, 1976: for a review see Spetch, Wilkie, & Pinel, 1981; Urushihara 2004; Vogel, Castro, & Saavedra, 2004) that both excitatory and inhibitory responses could occur as the result of a backward conditioning procedure. Moreover, these two kinds of responses mainly depend on the number of conditioning trials (the fewer the number of conditioning trials, the more excitatory the CS+ becomes; the greater the number of conditioning trials, the more inhibitory the CS+ becomes) and the “surprise” caused by the US (e.g., if US would be presented with CS-, which is never associated with US). Cole and colleagues (1999) for example suggested that temporal contiguity between CS+ and US is important in determining excitatory or inhibitory conditioned responses. They claimed that after few US-CS+ pairings (i.e., 4), the associative value of the backward CS+ is expressed as behavioural excitation,

whereas following many US-CS+ pairings (i.e., 96), a backward CS+ supported inhibitory behaviors. Therefore, it is possible to suppose here that the backward association between US and CS+ turned out in a potentiation of the startle amplitude (i.e., an excitatory conditioned response) because of the restricted number of US-CS+ pairings in this experiment.

A further consideration about the conditioned startle responses in this experiment may accord to "sometime opponent process" (SOP) model of Wagner (1981). Similarly to the opponent-process theory, this model conceives two states: A primary (A1) and a secondary (A2) state. When a stimulus is presented, it prompts an organism from an inactive state into a primary state (A1), this state will subsequently "decay" into a secondary opponent state (A2). Accordingly, excitatory conditioning occurs when elements of CS+ and elements of US are both in the A1 state. While, inhibitory conditioning occurs when elements of CS+ are in A1 state and elements of US are in A2 state. Possibly, the excitatory backward conditioning in this study may be due to the association between a memory trace of the just presented US and the current CS+. Accordingly, Romaniuk and Williams (2000) found that different components of a backward CS+ become either excitatory or inhibitory depending on the components' temporal proximity to US. The authors backward conditioned several groups of rats using an electric shock as US and delivering CS+ (a white noise of 10 dB) with different interstimulus intervals (ISI). When the US-CS+ interval was 0 s that is US corresponded with CS+ onset, the initial part of the backward CS+ acquired excitatory properties; in contrast, a 3 s US-CS+ interval supported inhibitory conditioning along the entire CS+. Romaniuk and Williams concluded that delivering CS+ exactly after US offset, the memory trace of US is still largely in an excitatory state (A1) as well as CS+ and therefore both US and CS+ are in state A1, the conditioned response is excitatory. When, however, there is a temporal gap of few seconds (i.e., 3 s) between US and CS+ the primary state of US is decayed into a refractory state (A2). US is thereby in state A2 and CS+ is in state A1 and consequently the conditioned response would be inhibition. Interestingly, these observations seem to conform to the excitatory responses after backward conditioning. In fact, the aversive US here was delivered just before the CS+ and probably both US and CS+ were still in state A1.

Nevertheless, the excitatory response in this study is in contrast to excitatory backward conditioning shown by Tanimoto et al. (2004), Cunningham et al. (2002) and Salvy et al. (2004). In fact, backward CS+ induced an approach response despite its association with an aversive US. Again, such discrepancy could accord to the SOP model. Thus, backward associations would develop excitatory learning to the CS+ if ISI is short and inhibitory learning if ISI is long. Consistently, Tanimoto et al. (2004) reported that fruit flies approached

backward conditioned odor when the shock (US) was presented at least 32-42 s before. Thus, the flies did not show conditioned approach when the interval between US and CS+ was too short. Similarly, the rats in Cunningham's study (2002) received an intragastric infusion of ethanol (US) immediately before exposure to the grid floor (CS+). But this operation lasted 30-45 s and the rats stayed in the chamber for 5 minutes. It may be that the effect of the ethanol could already have disappeared after 5 min. in the chamber; therefore the conditioned preference can be attributed to an association with the ethanol's refractory state (A2). As the SOP theory claims, when US is in the refractory state and CS+ is paired with it, CS+ becomes a conditioned inhibitor.

Consistently with the implicit negative valence, participants reported explicit negative valence and high arousal level for the shape (CS+) associated with the electric shock (US) compared to the control shape after both forward and backward conditioning. Therefore based on these results, it is possible to conclude that CS+ became more negative and aversive after both forward and backward associations when the aversive unconditioned event was presented just after or before the conditioned stimulus.

### 2.2. Forward and Backward Conditioning, Delay vs. Trace (Experiment 2)

According to the results of Experiment 1, the temporal gap between US and CS+ in the backward conditioning should have been too short to induce inhibitory or opponent responses. Therefore, I modified the interval between CS+ and US (i.e., ISI) in order to test if backward conditioning with long ISI would induce inhibition of the startle response. A forward trace conditioning paradigm was applied with a temporal gap between CS+ and US of 14 s. Also, backward conditioning was used and US was delivered few seconds before CS+. Finally, a forward delay conditioning was used, in which US was delivered at the offset of CS+. All things considered, there was a *"long" backward conditioning*, which should induce inhibitory responses because US should be in a refractory state (A2) while CS+ should be in a primary state (A1); a *"long" forward conditioning* which should be the comparison paradigm for the "long" backward conditioning and a *"short" forward conditioning* which should ensure that CS+ becomes a threatening signal and therefore aversive when it precedes an aversive US. Furthermore, the intensity of the painful US was increased by 1 mA referred to the individual pain threshold of the participants since strong painful stimulations modulate physiological reaction more efficiently (Lissek, Pine, & Grillon, 2006).

In summary, a between-subjects design was used here as in Experiment 1. The procedure was almost the same as in Experiment 1, with the modification of the ISI in both "long"

backward and traces forward conditioning, and the increased intensity of US. The hypothesis did not change for this study. Thus, I expect that the forward conditioning would induce negative implicit valence of CS+ (i.e., startle amplitude would be potentiated in the presence of the forwards CS+ compared to a control neutral stimulus), whereas backward conditioning would induce positive implicit CS+ valence (i.e., startle amplitude would lessen in the presence of the backward CS+ compared to a control neutral stimulus). Consistently, explicit valence ratings should indicate a more negative and more positive CS+ valence after forward and backward conditioning respectively.

### 2.2.1. Method

2.2.1.1.*Participants*

101 healthy volunteers participated in the study. The sample included 68 women and 33 men ranging in age from 18 to 43 years old (mean age = 23.2 years; SD = 4.6). Participants were free of neurological, psychiatric or chronic pain diseases. Prior to the experiment, they were informed of the experimental procedure and that they would receive some electric stimuli.

The sample was divided into three groups. One group received the US at the offset of the CS+ (forward conditioning: 34 participants, 19 of which were female), one group received the US 6 s before CS+ onset (backward conditioning: 33 participants, 25 of which were female) and the third group received the US 6 s after CS+ onset (control conditioning: 34 participants, 19 of which were female).

*2.2.1.2. Procedure*

The procedure has already been described in the first experiment with two exceptions. The intensity of the shock was increased by 1 mA after assessment of individual pain threshold and the time between CS+ onset and US onset (ISI) was 8 s for forward conditioning, -6 s for backward conditioning and 14 s for control conditioning.

*2.2.1.3. Data Analysis*

Blink amplitude was assessed 3 x 3 mixed model ANOVAs with between-subjects factor conditioning (forward, backward, control) and within-subjects factor stimulus (CS+, CS-, NEW). Subjective ratings for the valence and arousal were separately compared with 3 x 3 x 2 mixed model ANOVAs with between-subjects factor conditioning (forward, backward, control) and within-subjects factors stimulus (CS+, CS-, NEW) and time (before, after conditioning).

The α level was set at .05 for all statistical tests. Greenhouse-Geisser corrections (GG-ϵ) were used for the main effects and interactions involving factors with more than two levels.

### 2.2.2. Results

*2.2.2.1.Subjective Ratings*

The repeated measures ANOVA for the valence did show a significant main effect of stimulus ($F(2, 196) = 12.83$, $p = .000$, GG-ϵ = 0.88). Thus, CS+ valence was generally rated more

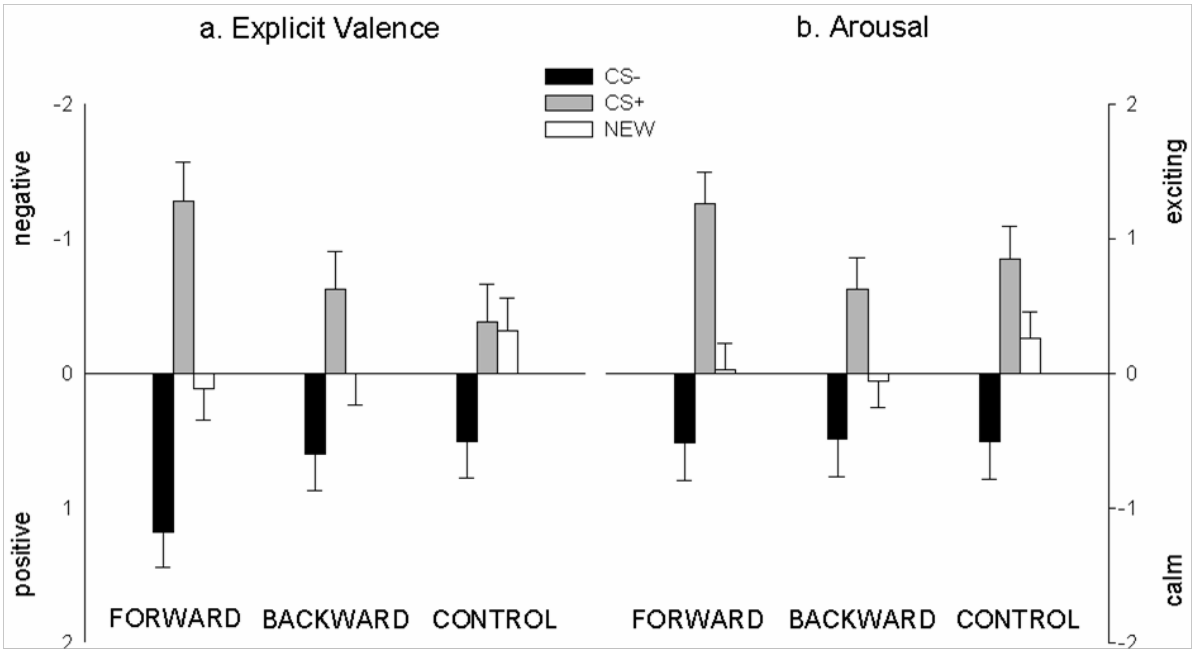



*Figure 2.6.* Explicit valence and arousal assessed with subjective ratings after forward, backward and control conditioning in Experiment 2.

Stimuli were rated before and after training for valence and arousal. Bars (with standard errors) represent the subjective scores of the valence (on the left side) and the arousal (on the right side) of the visual stimuli after forward, backward and control conditioning. Black bars represent CS- ratings; gray bars CS+ ratings and white bars the ratings for the NEW stimulus. Values on the y-axis are differential values between participant ratings and the neutral (i.e., 5) **a**. Ratings of the valence were consistently negative after training phase independently from event timing. This indicates that stimuli reinforced during conditioning were rated more negatively compared to their initial ratings. **b.** Ratings of the arousal were consistently rated high arousing compared to their initial arousal.

negative than CS- and the NEW stimulus. No significant main effect of time was found ($F(1, 98) = 3.38$, $p = .069$). But a significant interaction of Stimulus x Time was found ($F(2, 196) = 22.43$, $p = .000$, GG-ϵ = 0.91) (Figure 2.6a) indicating that the visual shapes significantly

changed their valence after the conditioning. Follow up tests indicate that after forward conditioning, CS+ valence was rated significantly more negative ($t(33)= -3.66$, $p = .001$) and CS- valence more positive ($t(33) = 3.53$, $p = .001$) both compared to the NEW stimulus. CS- valence significantly differed from NEW valence after both backward ($t(32)= 2.27$, $p = .030$) and control ($t(33)= 2.96$, $p = .006$) conditioning. CS+ valence did become significantly more negative after forward ($t(33)= 5.55$, $p = .000$) and backward ($t(32== 2.30$, $p = .028$) conditioning, but not after control ($t(33)= 1.23$, $p = .226$) conditioning compared to its initial valence. In absolute terms, both forward and backward CS+ acquired negative valence since these valence ratings after the training phase did significantly differ from 5, the neutral value (FORWARD: $t(33) = -4.65$, $p = .000$; BACKWARD: $t(32) = -1.95$, $p = .060$, tendent) this was not the case for the CONTROL CS+ ($t(33) = -1.4$, $p = .172$). Since the interaction Stimulus x Time x Conditioning resulted ($F (4, 196) = 4.12$, $p = .004$, GG-$\epsilon = 0.91$), comparison between conditioning indicated that forward CS+ was rated significantly more negative than the control CS+ ($t(66) = 2.34$, $p = .023$) but backward CS+ ($t(65) = 0.54$, $p = .590$) was not.

The repeated measures ANOVA of arousal did show a significant main effect of stimulus ($F (2, 196) = 13.31$, $p = .000$, GG-$\epsilon = 0.85$). Thus, CS+ was generally rated more arousing than CS- and the NEW stimulus. No significant main effect of time was found ($F(1, 98) = 1.43$, $p = .235$), but the Stimulus x Time interaction resulted significant ($F(2, 196) = 16.69$, $p = .000$, GG-$\epsilon = 0.91$) (Figure 2.6b). No significant Stimulus x Time x Conditioning interaction was found for arousal ratings ($F (4, 196) = 0.56$, $p = .673$, GG-$\epsilon = 0.91$). Follow up tests indicated that shape arousal did not differ among stimuli before conditioning and was rated as neutral. Forward CS+ ($t(33) = 3.53$, $p = .001$), backward CS+ ($t(32) = 2.27$, $p = .030$) and control CS+ ($t(33) = 2.26$, $p = .031$) were rated more arousing than the NEW stimulus. Moreover, arousal ratings did significantly change after the training phase. Indeed, both forward and control CS+ acquired significantly higher arousal compared to their initial rating (forward: $t(33) = -3.14$, $p = .004$; control: $t(33) = -3.06$, $p = .004$), but backward CS+ did not significantly change its arousal ($t(32) = -1.26$, $p = .216$). In absolute terms, the FORWARD, the BACKWARD and the CONTROL CS+ acquired higher arousal since the arousal ratings after training did significantly differ from 5 (FORWARD: $t(33) = 4.91$, $p = .000$; BACKWARD: $t(32) = 2.03$, $p = .051$; CONTROL: $t(33) = 4.4$, $p = .000$).

*2.2.2.2. Startle Amplitude*

The analysis of the eye blink responses did not show a main effect of stimulus ($F (2, 196) = 0.57$, $p = .566$) (Figure 2.7.). However, a significant difference was found between forward

and backward conditioning (F (4, 196) = 3.49, $p$ = .009). Separate follow up tests indicated that startle responses to forward trained CS+ were significantly higher ($t$(33) = 2.91, $p$ = .006) and startle responses to backward trained CS+ were significantly lower ($t$(32) = -2.1, $p$ = .044) compared to the mean (0). No significant difference among visual stimuli was found after control training ($t$(33) = -0.13, $p$ = .894).

*2.2.2.3. Contingency Awareness of CS-US Association*

The contingency data showed significant main effect of stimulus (F (1, 96) = 143.25, $p$ = .000) indicating that the contingency awareness between CS+ and CS- did significantly differ. A significant main effect of time (F (1, 96) = 5.43, $p$ = .022) was found indicating that contingency awareness ratings did significant change after extinction compared to conditioning. Finally, a significant Stimulus x Time interaction (F (1, 96) = 19.71, $p$ = .000) and a Stimulus x Time x Conditioning interaction (F (2, 96) = 4.59, $p$ = .013) were found.

Follow up tests indicated that the stimulus contingency was significantly higher for CS+ compared to CS- after both forward ($t$(33) = -15.63, $p$ = .000), backward ($t$(32) = -6.59, $p$ = .000) and control ($t$(33) = -5.34, $p$ = .000) training. Anyway, such ratings changed after the extinction phase that is the contingency awareness significantly decreased after extinction (forward: $t$(33) = 4.09, $p$ = .000). However, differences between CS+ and CS- were still significantly marked after extinction (forward: $t$(33) = -4.52, $p$ = .000; backward: $t$(32) = -5.55, $p$ = .000; control: $t$(33) = -4.35, $p$ = .000).

Contingency awareness did significantly differ between forward conditioning compared to control conditioning ($t$(64) = 2.50, $p$ = .015). There was, however, no significant difference among trainings after the extinction phase. Contingency awareness between backward and control conditioning did not significantly differ.

### 2.2.3. Discussion

These findings show that event timing modulates the implicit valence of a conditioned stimulus in an opponent manner, but not its explicit valence. Thus, startle responses are potentiated in the presence of a stimulus *signaling* an aversive event indicating implicit negative valence, but are attenuated in the presence of a stimulus *following* an aversive event indicating implicit positive valence. On the contrary, subjective ratings for the conditioned stimulus valence did not show such an opponent modulation; in fact both stimuli are rated as “emotionally” negative.

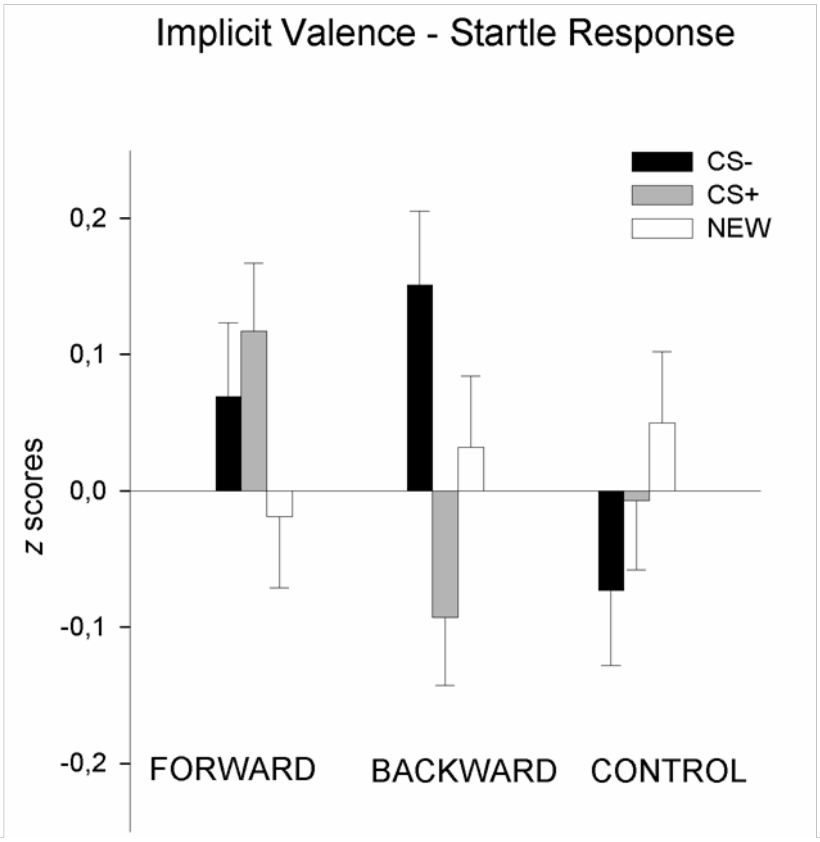



*Figure 2.7.* Implicit valence assessed with startle response after forward and backward conditioning in Experiment 2.

Bars represent mean (with standard errors) startle amplitudes in *z*-scores at the presence of the visual stimuli (in black CS-, in gray CS+ and in white NEW) after forward (on the left side), after backward conditioning (in the middle) and after control conditioning (on the right side). Positive values indicate startle response potentiation, negative values startle attenuation. During test phase, startle responses were elicited by a loud white noise in the presence of either, CS-, CS+ or the NEW stimulus. When electric shock (US) briefly followed CS+, startle responses were potentiated indicating implicit negative valence. When, however, US preceded CS+, later on startle responses were attenuated in the presence of that backward CS+ indicating implicit positive valence. When ISI was really long (control conditioning), no startle modulation was found in the presence of CS+.

Timing dependent bidirectional modulation of human startle behavior seems to conform to dual-process theories. The opponent-process theory of acquired motivation (Solomon, 1980), for example, suggests that an aversive stimulus such as a painful electric shock generates two opponent processes: An initial negative affect and an after-process entailing the opposite state that is positive affect. In other words, the stimulus presented *after* the aversive event attenuates fear responses and such opponent modulation might be explained through the

association between backward CS+ and the appetitive after-process. Corroborating this interpretation, previous studies reported that pain relief may share reward circuits (Brischoux, Chakraborty, Brierley, & Ungless, 2009; Leknes, & Tracey, 2008; Seymour, et al., 2005). Besides, our results may also conform to Dickinson's hypothesis (1979) or to Seligman's (1971) "safety signal" hypothesis. In his model Seligman (1971) assumed that where CS+ presence reliably predicts US, its absence reliably predicts US absence. Assuming that US is an aversive or negative event, then its absence would be appetitive or positive. Consistently, the backward CS+ following an aversive US predicted the absence of the aversive US and startle responses are attenuated.

Notably, the results concerning startle modulation may also be explained on the basis of spike timing dependent plasticity (STDP) (Drew, & Abbott, 2006). That is, the temporal sequence of two inputs determines whether synapses are potentiated or depressed. This process can conceivably act in behaviorally relevant time scales (Drew, & Abbott, 2006; Abbott, & Nelson, 2000), in particular if it operated in the amygdala and/or in the dopamine neurons of NAcc, both structures relevant for startle response modulation (Brischoux, et al., 2009; Schneider, & Spanagel, 2008; Davis, 2006). Additionally, converging evidence indicates that potentiation or depression of synaptic firing in both amygdala (Delgado, et al., 2006; Kim, et al., 2006) and NAcc (Wise, 2004) are underlying mechanisms of associative memory.

Importantly, these results revealed an event timing specific dissociation between implicit and explicit valence. A previously neutral stimulus presented briefly after an aversive event acquires positive implicit valence but is explicitly evaluated as negative. Curiously, paradoxical human behaviors like approaching stimuli that are explicitly evaluated as negative or dangerous (e.g., rollercoaster ride, bungee jumping) are maintained. Contemporary approaches assert the importance of classical conditioning in understanding the etiology and maintenance of anxiety disorders (Mineka, & Oehlberg, 2008) or the pathological behaviors resulting from drug addiction (Koob, et al., 2001; Weiss, 2005). For example, experiencing a panic attack in a specific situation may lead to fear and to avoidance of this situation and patients are frequently characterized by being attracted to "safety" stimuli (e.g., a physician, an ambulance) (Himadi, 1967). Similarly, drug withdrawal is often associated with negative feelings, anxiety, irritability and depression that are followed by relief when the person takes the drug. As a consequence, this may initiate a circuit of relapses, the underlying mechanisms of which are still unclear (Koob, et al., 2001; Weiss, 2005). Therefore, more detailed analyses of the reward-like after-effects of aversive events are necessary to comprehensively

understand the impact of aversive or traumatic events on pathological human behaviors, including its potential modulation by genotype (see Yarali, Krischke, Michels, Saumweber, Mueller, & Gerber, 2008).

To conclude, the present study shows that event timing plays a crucial role in the acquisition of the positive or negative implicit valence of a conditioned stimulus despite its association with an aversive event. Moreover, the responses of the impulsive, implicit system dissociate from the responses of the reflective, explicit system supporting the antagonistic interplay between the two systems, especially when contrasting inputs are on hand (i.e., the "appetitive" pain termination and the aversive US). Further studies should then clarify the mechanisms behind the opponent processes involved in event timing.

### 2.3. General Discussion

According to dual-process theory (Bechara, 2005; Strack, & Deutsch, 2004), humans have explicit and implicit cognition. The impulsive and reflective systems differ in how they activate behaviors. The reflective system is more likely to control behaviors if the necessary cognitive capacity is available. Conversely, the impulsive system is more likely to have the upper hand under a strong deprivation of basic needs or under a motivational orientation that facilitates the execution of the behavior. Between these two systems there is a synergic and antagonist interplay that determines which behavior the organism is going to act out. If the two systems cooperate to the activation of the same behavioral schema, such behavior is facilitated. However, if they compete, then they may activate incompatible schemata. And such antagonist activation may be accompanied by a feeling of conflict and temptation. Notably, these two systems influence each other, but at the same time they are independent from each other.

Here, the dissociation between verbal reports and defensive responses seems to conform to the impulsive-reflective model. In fact, event timing seems to influence the impulsive but not the reflective system. Independently from both the time sequence and also from the temporal gap between CS and US, participants rated the stimulus associated with the aversive event as "emotionally" negative. Hence, no matter whether the shock was delivered just before CS+ or just after CS+; a few seconds before CS+ or a few seconds after CS+, this stimulus that was perceived as being associated with the painful shock was also experienced as negative. Differently, the impulsive system seems to be strongly influenced by event timing. Indeed, the implicit tool of the valence (i.e., the startle response) indicated opponent responses according to the temporal sequence between CS and US. Thus, if the aversive US just followed the CS+

(ISI = 8 s), fear responses were potentiated in the presence of this stimulus indicating implicit negative valence and increase of fear. Hence, I suppose that CS+ primes the organism to defensive responses because an aversive event is coming and consequently forward CS+ becomes a threat signal. Moreover, such priming suggests that the (impulsive) defensive motivational system activated by forward CS+ should mainly involve activation in the amygdala. When, however, the painful shock followed the CS+ with a really long temporal gap (i.e., ISI = 14 s), no fear responses has been shown confirming that both the temporal contiguity and the contingency are two important aspects in learning how to predict threats. Finally, when the shock preceded the CS+, fear responses were either potentiated or attenuated. Thus, potentiation of fear responses was observed when the shock was delivered just before the shape onset (ISI = -0.2 s) and attenuation of fear responses was observed when the shock preceded the shape by few seconds (ISI = -6 s). These last findings indicate implicit positive valence and activation of the (impulsive) appetitive motivational system. So backward conditioning seems to induce both fear and no-fear. How is this explainable?

A suitable theory for these results seems to be the “sometime opponent process” (SOP) model (Wagner, 1981). As I already clarified in the introduction, the SOP theory incorporates a US memory trace that supports both excitatory and inhibitory backward conditioning. Additionally, Romaniuk and Williams (2000) claimed that a backward CS+ may have different components which can become excitatory or inhibitory depending on their temporal proximity to US. In this study, the rats received extensive conditioning of two auditory CSs, one was backward paired with an electric shock (US) and the other was forward paired. The interstimulus interval (ISI) between CS+ and US lasted either 0 s or 3 s. During the test phase either the forward or the backward CS+ was presented during a lever press response. The authors found that the initial part of a backward CS+, which occurred in direct contiguity with the US, had an “excitatory” aversive effect. The response was indeed punished and animals suppressed the lever press response. Similar punishing effect was observed in the presence of the forward CS+. However, such punishing effect was not observed when the shock preceded the CS+ with a temporal gap of 3 s and the animals increased lever press responses. These results allow the supposition of similar effects of timing on the backward CS+ in the two startle-studies here. When backward CS+ was presented in direct contiguity with US (ISI = -0.2 s), startle responses were potentiated (excitatory) in the presence of such backward CS+ during the test phase (Figure 5.). When, however, a delay between the US and the backward CS+ presentation was applied (ISI = -6 s), startle response was attenuated (inhibitory) in the presence of such backward CS+ (Figure 7.). In other words, the closer the temporal proximity

between the shock and the shape is, the more the fear responses are increased. In conformity with the SOP theory, this "excitatory" conditioning might be due to active state A1 of both the US memory trace and the backward CS+ presence. Contrarily, a longer delay between the two events lets us suppose that the backward CS+ is in a primary state (A1), whereas the aversive US is already in a refractory state (A2), as a consequence, the association between these two events conducts to an inhibitory conditioning. The clarification of the nature of this refractory state A2 still remains. In fact, whilst on the one hand the SOP theory makes it clear that the association between two events induces an inhibitory conditioned response when one event is in state A1 and the other one in state A2, it does not, on the other hand, explain the properties of such an inhibitory response. According to these results, the attenuation of startle amplitude after "long" backward conditioning leads us to suppose that the after-process (or the refractory-state as we would want to call it) has a positive valence and therefore I would expect an activation of the appetitive system. I am going to clarify this hypothesis in the next chapter.

Curiously, explicit tools for valence did not bear out the implicit tools. Thus, even if the participants showed (impulsive) positive implicit valence, they reported (reflective) explicit negative valence. A better understanding of this opposite and dissociative interplay between the impulsive and reflective system may lead to the elucidation of anxiety disorders (Bouton, et al., 2001; Himadi, 1987; Mineka, & Oehlberg, 2008) or the mechanisms behind drug addiction (Koob, et al., 2001; Weiss, 2005).

A last consideration regards the contingency awareness. Contingency awareness is defined as the participant's knowledge about the association between CS+ and US (Devriese, Winters, Van Dienst, & Van den Bergh, 2004). Fear conditioning may occur unconsciously through automatic mechanisms that are independent of awareness and not require cognitive input, and consciously through cognitive and reflective mechanisms (Hamm, & Weike, 2005). The automatic mechanism would likely be adaptive and may allow animals to be biologically prepared for danger relevant cues (Öhman, & Mineka, 2001; Seligman, 1971). The large body of neuroanatomical evidence from animals supported the crucial role of the amygdala in this "quick-and-dirty" learning mechanism (Davis, 1998; LeDoux, 1995). However, humans are complex organisms who can also think about event associations. And it is just this human ability that might have a critical role in the opposite results after "long" backward conditioning. Thus, participants showed contingency awareness of the association between CS+ and the aversive US. I would think that the explicit conditioned responses (subjective ratings) were especially influence by such knowledge and therefore the impulsive and the

reflective system might have acted in an antagonist fashion. Furthermore, there is daily evidence of opponent behavior towards dangerous events. It is enough to think about extreme sports like parachuting, which are claimed to be terrifying and dreadful but are repeated again and again anyway.

In summary, the conditioning procedures I used were all effective in inducing conditioned responses. And to my knowledge, this is the first evidence showing the opponent modulation of event timing in humans. However, much more work should be done in trying to unravel both the mechanisms and the neural correlate of such opponent reactions. Only then will it be possible to know if and how event learning plays a role in the etiology of emotional disorders.

## 3. The Neural Circuits Underlying Event Learning

Event timing modulated fear responses in an opposite fashion. Thus, startle was potentiated by stimuli that preceded an aversive event and was attenuated by stimuli that followed an aversive event. Starting from these findings, the aim of this study is to investigate the neural correlates underlying the opponent modulation by event timing of the conditioned startle responses.

Functional magnetic resonance imaging (fMRI) is currently the mainstay of neuroimaging in cognitive neuroscience. Its principal advantages lie in its noninvasive nature, ever increasing availability, relatively high spatiotemporal resolution and its capacity to determinate the entire network of brain areas engaged when subjects undertake particular tasks. One disadvantage is that it measures a surrogate signal whose spatial specificity and temporal response are subject to both physical and biological constraint. In human neuroimaging, the blood-oxygen-level-dependent (BOLD) contrast mechanism is the mainly used, namely the differences in blood oxygenation depending on the neural activation of a region while executing a particular task (Büchel, et al., 1998). The optimal prerequisite for studying the neurobiology of classical conditioning in humans using functional neuroimaging is met by event-related fMRI technique (Büchel, et al., 1998). In fact, event-related design allows researchers to study evoked hemodynamic activity in single stimulus categories (e.g. to CS+ or to CS-) and to separate responses related to acquisition and extinction of conditioning. In simple terms, this technique is analogous to the recording and the analysis of event-related potentials in electrophysiology, where different stimuli are presented and the responses sampled repeatedly over time, but without having its temporal resolution (Büchel, & Dolan, 2000).

Human fMRI studies (Büchel, et al., 1998; Cheng, et al., 2003; for a review see Delgado, et al., 2006; LaBar, et al., 1998; Phelps, et al., 2001; Tabbert, et al., 2006) supported previous animal findings (Davis, 2006; LeDoux, 1995) showing the crucial role of the amygdala in Pavlovian fear conditioning. Further regions such as the insula, ACC and OFC play a role in conditioned fear expression too (Büchel, et al., 1998; Milad, et al., 2007; Phelps, et al., 2001). LaBar and colleagues (1998), using a single-trial fMRI to study acquisition and extinction phases separately, found increased signal in the amygdala evoked by the CS+ referred to CS-. In this study, CSs were geometrical shapes and the US an aversive electric shock. Amygdala activation has also been to be found enhanced during either the initial acquisition or extinction trials but not during the late trials. This suggests that amygdala activation decreases over time, i.e. habituated. Similarly, Büchel and colleagues (1998) found a greater signal in the

amygdala in response to CS+ compared to that evoked by CS- when CS+ was associated with an aversive US. In any case, amygdala activation habituated rapidly over time in this study as well. Hence, these two studies highlighted the important role for the amygdala in fear learning especially during the early acquisition phase and early extinction. These results are also consistent with the idea that the amygdala serves as a rapid subcortical information processing pathway for behaviorally relevant (e.g. dangerous) stimuli (LeDoux, 1995). Interestingly, Phelps and colleagues (2001), using the quite different paradigm of fear conditioning, i.e. instructed fear protocol, found greater activation in the amygdala by threat cues compared to safe cues. During instructed fear, subjects did not actually receive an aversive US like an electric shock, but they were told that such an aversive event might occur in conjunction with a particular neutral stimulus. As I reported above, participants showed greater activation in the amygdala in response to a stimulus linked to an instructed threat (CS+) than to a stimulus linked to an instructed absence of threat i.e. safe (CS-). Amygdala activation habituated over time in this study as well as in Büchel's and LaBar's studies. A further study pointed out the fundamental role of the amygdala in both fear learning and fear expression (Tabbert, et al., 2006). Tabbert and colleagues (2006) highlighted the automatic nature of fear acquisition distinguishing the cortical regions involved in automatic fear learning (i.e. the amygdala) and those involved in more complex, cognitive processes in fear learning. Indeed, they found amygdala activation in response to the stimulus associated with an aversive stimulus in those participants who could correctly verbalize the contingency awareness between CS+ and US as well as in those participants who could not verbalize the association.

However, amygdala is not the only structure implicated in fear conditioning, but ACC and insula also have a fundamental role. It has been suggested that the insula and anterior cingulate cortex provide a route through which nociceptive inputs are integrated with memory traces to allow appropriates responses (Büchel, et al., 1998). Moreover, Phelps and colleagues (2001) found great insula activation in response to the instructed threat and the author interpreted this result as being a consequence of the possible function of the insula in the cortical conveyance of fear representation to the amygdala. In another two studies using fear conditioning, Knight (2004) and Milad (2007) proposed that ACC could be mainly involved in the expression of fear response. Supportively, both ACC and insula integrate the sensorial inputs from the thalamus with the cognitive inputs from the frontal cortex and based on this processing allow the organism to respond to the forthcoming events appropriately.

To my knowledge, only animal studies have investigated and reported opponent conditioned responses to a stimulus associated with an aversive unconditioned event depending on the time sequence between them. In other words, mice presented conditioned

place preference (CCP) for a chamber that was backward associated with an unpleasant ethanol injection (Cunningham, et al., 2002), but showed conditioned place aversion (CPA) for a chamber that was forward associated with the unpleasant US. Or rats demonstrated less suppression of drinking a sweet solution that was backward associated with the running-wheel (i.e. the aversive US) compared to the forward conditioned group (Salvy, et al., 2004). Finally, fruit flies flew toward a backward conditioned odor (i.e. approach response) referred to the avoidance responses to a forward conditioned odor (Tanimoto, et al., 2004). These findings indicate that backward association with an aversive event may induce appetitive responses. In addition to this, the study described in the second chapter of this thesis showed that fear responses were attenuated by the backward conditioned stimulus suggesting again appetitive response to a stimulus backwardly associated with a threat. However, there is no evidence that indicates which cortical structures underline such an appetitive response after backward conditioning. In line with previous findings, I would expect that the aversive motivational system is activated by the forward CS; whereas the appetitive motivational system is activated by backward CS. Therefore, amygdala, ACC and insula would be strongly activated in response to forward CS+ referred to a control neutral stimulus. However, because of the lack of evidence in humans regarding backward conditioning, I focused on the appetitive conditioning to find those regions of interest (ROI) that might be involved after backward association.

Contrarily to fear conditioning, appetitive conditioning is a form of associative learning in which a neutral stimulus acquires a new motivational significance through its association with a rewarding event (Martin-Soelch, Linthicum, & Ernst, 2006). A reward is an object or an event that generates approach behaviors and consumption, produces learning of such behaviors (Schultz, 2007). There are several evidences showing that the association between a neutral stimulus with both primary appetitive reinforcers (e.g. food) and secondary reinforcers (e.g. money) induced activation in the human mesolimbic dopaminergic system (Gottfried, et al., 2002; Knutson, Fong, Adams, Varner, & Hommer, 2001b; O´Doherty, et al., 2004). Using a classical conditioning paradigm in which pictures of neutral faces (CS) were associated either with an unpleasant odor (aversive US) or with a pleasant odor (appetitive US), Gottfried and colleagues (2002) found greater activation in the nucleus accumbens (NAcc) in response to the appetitive-CS referred to the aversive-CS. This result implies therefore that NAcc activation would be specifically related to the learning processes of an olfactory reward. O´Doherty and colleagues (2004) found activation in the striatum, which includes the NAcc, in response to a stimulus associated with a fruit juice. And Knutson and LeMoal (2001), using a monetary incentive delay task (MID), found that the NAcc was primarily

recruited by the anticipation of monetary reward. Such activation was however suppressed when the anticipated reward was not obtained (i.e. punishment). Consistently, O´Doherty and colleagues (2003) found greater activation in the ventral striatum to rewarding versus punishing stimuli again supporting the role of the striatum, in particular of its ventral part, in reward prediction. Therefore, these evidences suggest that striatum, in particular the NAcc, may be involved in reward anticipation and in learning processes with rewarding stimuli. Moreover, if the amygdala is the main cortical substrate of the defensive system, the striatum might be considered the crucial region in animal and human brain underlying the appetitive system.

Interestingly, it has been found that the termination of a painful stimulus entails rewarding properties. Indeed, Seymour and colleagues (2005) found that relief from a heat pain (US) involved a reward-like learning signal. In their study, the authors used three kinds of visual cues: a relief-related cue followed by decrease of the temperature, a pain-exacerbation-related cue followed by increase of the temperature and a third control cue followed by no change in the temperature. Both aversive and appetitive systems were simultaneously involved in encoding appropriate goal-directed predictions of outcomes. Thus, the ventral striatum was strongly activated by the relief-related cue, whereas the ACC was activated by the pain-exacerbation cue. Furthermore, if the dopaminergic system is involved in reward processing, on the other hand, there are findings showing its involvement in processing of aversive events. Menon and colleagues (2007) gave amphetamine to one experimental group of participants and haloperidol to a second one. Amphetamine is an agonist drug for the dopamine, whereas the haloperidol is an antagonist for the DA. Both groups underwent discriminatory conditioning in which a visual stimulus (CS) was associated with a painful electric shock (US). Participants, who took amphetamine, showed great activity in the striatum implicating the role of the dopamine in this fear conditioning paradigm. And such activation was not found in participants, who took haloperidol. Moreover, between-subjects comparison indicated greater activity in the striatum and in the midbrain in the amphetamine-group compared to the placebo-group. Hence, these results once again underline the involvement of the striatum (and possibly of the NAcc) and the dopaminergic system in processing salient stimuli. Therefore, based on this evidence, I expect great activation in the striatum to be induced by the backward conditioned stimulus associated with a painful electric shock compared to a neutral control stimulus.

## 3.1. Method

### *3.1.1. Participants*

A total of 28 volunteers (15 female; mean age = 22.68 years; SD = 2.72; range = 19-29 years) participated in the study. The sample was divided into two groups of 14 participants. One group underwent a forward conditioning procedure and the other group underwent a backward conditioning procedure. Participants were students at the University of Würzburg or young citizen of Würzburg. Participants were informed about the procedure though the conditioning schedule was not explained until the experiment was finished. They were just told that they would sometimes receive a painful electric shock.

Participants were free of neurological, psychiatric and chronic pain diseases.

### *3.1.2. Stimulus Material*

The *unconditioned stimulus* (US) was a 200 ms cutaneous electric pulse stimulation with 500 Hz frequency and was applied to the left index finger, delivered via surface bar electrodes. The electrodes consisted of two durable gold-pasted stainless steel disk electrodes with 9 mm diameter, 30 cm spacing, with an impedance of 5 Ω. The electric stimulation was generated by a constant-current stimulator (Digitimer DS7A) supplying a maximum of 400 V and 5 mA. The intensity of the US was set individually using the same procedure for the pain threshold that I used for the previous studies (for more details see Chapter 2).

Three pictures of simple geometrical shapes, a square, a circle and a triangle, served as visual *conditioned stimuli* (CS) in a differential conditioning paradigm. Similarly to the startle-study, visual stimuli could function as a conditioned stimulus associated to the US (CS+), as a conditioned stimulus never associated with the US (CS-) or as a neutral control stimulus (NEW) only presented during the test phase. Stimuli were counterbalanced among participants. Visual stimuli were displayed via compatible goggles (VisuaStim, Magnetic Resonance Technologies, Northridge, CA) on a black background. All visual stimuli were yellow in color with identical luminance and 10 s in duration. Presentation (Version 11.1; Neurobehavioral Systems Inc.) was the software used for presenting stimuli on the display.

### *3.1.3. Procedure*

The procedure for this study was really similar to the previous startle-study except that no startle probes were delivered during the test phase.

Upon arrival in the laboratory, participant signed an informed consent that was approved by the ethics committee of the ‘Deutsche Gesellschaft für Psychologie’ (DGPs). The German version of the State-Trait Anxiety Inventory (STAI - Laux, Glanzmann, Schaffner, &

Spielberger, 1981) was collected. Then their pain threshold was assessed. Initially, participants underwent an anatomical scan lasting approximately 6 min in the absence of visual and electric stimulation. After this scan, the experiment began.

The c*onditioning* consisted of 16 trials of CS+ and 16 trials of CS-. The intertrial interval (ITI) lasted 20 s. Participants were divided into two experimental groups, one group underwent forward conditioning and the other group underwent backward conditioning. During forward conditioning, the onset of the US followed CS+ onset by 10 s (ISI = 10 s). During backward conditioning, the onset of the US preceded CS+ onset by 6 s (ISI = -6 s). Conditioned stimuli were presented with a random order with the only restriction that not more than two stimuli of the same category were presented in a row.

The *extinction* (i.e. the test phase) consisted in 10 trials of CS+, 10 trials of CS- and 10 trials of a NEW stimulus. No electric shock was delivered and ITI were 20 s in duration. Again, stimuli were presented in a randomized order with the restriction of no more than two consecutive presentations of the same stimulus.

Participants rated the valence and the arousal of the geometrical shapes before and after conditioning. During the *subjective ratings*, a visual analogical scale (VAS) ranging from 1 to 9 was presented on the display. One meant 'really unpleasant' for the valence and 'calm' for the arousal, 9 meant 'really pleasant' and 'exiting' respectively. The value 5 indicated neutral valence (either unpleasant or pleasant) and neutral arousal (either calm or exciting).

Finally, I also controlled the contingency awareness of the participants after both conditioning and extinction. In this case, a VAS ranging from 0% to 100% was presented on the display. 0 meant no association between the visual stimulus and the shock and 100 indicated association between CS and US.

### *3.1.4. Magnetic Image Resonance*

Brain images were acquired using a 1.5-T whole-body tomography (Siemens Avanto with a quantum gradient system) with a standard head coil and a custom-built head holder to reduce movement. The structural image acquisition consisted of 160 T1-weighted sagittal magnetization-prepared rapid gradient-echo imaging (MP-RAGE) 3D MRI sequence (MPRAGE, 1 mm slice thickness, TR = 2250 ms, TE = 3.93 ms, 8° flip angle, FOV = 250 mm, matrix = 256x256, voxel size = 1x1x1 mm). For functional imaging a total of 940 volumes for forward conditioning and 1004 volumes for backward conditioning were registered using a $T^*_2$-weighted gradient echo-planar imaging sequence (EPI) with 25 axial slices covering the whole brain (5 mm slice thickness; 1 mm gap, descending order, TA = 100 ms; TE = 40 ms, TR = 2500 ms, flip angle = 90°, field of view = 240x240 mm, matrix size =

64x64, voxel size = 3.1x3.1x5 mm). The orientation of the axial slices was parallel to the AC-PC line.

### *3.1.5. Image Preprocessing and Statistical Analysis*

Data were analyzed using a Statistical Parametric Mapping (SPM5, Wellcome Department of Cognitive Neurology, London) within MatLab 7.0 (Mathworks Inc., Sherborn, MA).

Realignment (b-spline interpolation), slice time corrections were performed (Ashburner, & Friston, 2003). To allow localization of functional activation on the participants´ structural MRIs, T1-scans were coregistered to each participant's mean image of the realigned functional images. The EPI images were subsequently normalized to the Montreal Neurological Institute (MNI) space using the normalization parameters obtained from the segmentation procedure (voxel size 2x2x2 $mm^3$) and spatially smoothed with an 8 mm full-width-half-maximum (FWHM) Gaussian kernel.

The experimental conditions were modeled by convolving stick functions with the canonical hemodynamic response function (HRF). The six movement parameters of the rigid body transformation, applied by the realignment procedure, were introduced as covariates. The voxel-based time series were filtered with a high pass filter (512 s time constant). In order to prevent specific processes implicated in the extinction, we considered the activation in response to the visual stimuli during the first extinction trials[10], i.e., we considered only the first five presentations of CS+, CS-, and NEW, respectively. For each participant, *t*-contrasts (CS+ > CS-; CS+ > NEW) were computed, but only for extinction. For a random effect analysis, the individual contrast images (first-level) were used in a second-level analysis. For the statistical analysis explorative whole brain as well as Region of Interest (ROI) analyses was used to enhance the statistical power concerning the structures of interest: the amygdala, the cingulate cortex (anterior and posterior), the insula, the striatum (ventral and dorsal) and the orbitofrontal cortex. Importantly, the hemodynamic responses to the visual stimuli were analyzed separately for forward and backward conditioning and only in a second moment were the two kinds of learning compared. The choice was mainly due because I wanted check whether the paradigms worked and also because there is no evidence about the neural circuits of the backward conditioning.

In order to investigate brain activity in relation to subjective ratings, valence and arousal ratings as well as contingency awareness a correlation analysis of estimated BOLD responses and subjective ratings was performed, verifying whether there was a relationship between the ratings and the cortical activation.

WFU Pickatlas software (Version 2.3, Wake Forest University, School of Medicine, NC) was used to conduct the Small volume correction with pre-defined masks in MNI-space (Tzourio-Mazoyer, Landeau, Papathanassiou, Crivello, Etard, Delcroix, Mazoyer, & Joliot, 2002; Maldjian, Laurienti, Kraft, & Burdette, 2003; Maldjian, Laurienti, & Burdette, 2004).

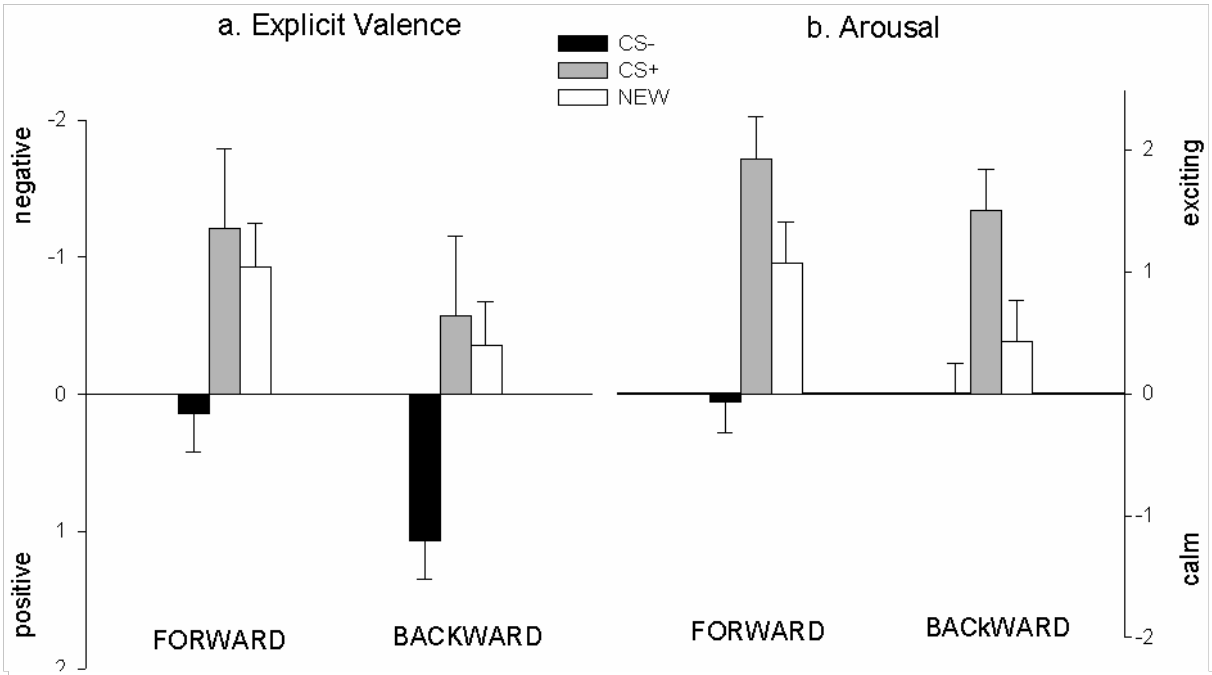



*Figure 3.1.* Explicit valence and arousal assed with subjective ratings after forward and backward conditioning.

Participants had to rate valence and arousal of the visual stimuli before and after training. Bars (with standard errors) represent the subjective scores after forward and backward conditioning. Black bars represent CS-; gray bars represent CS+ and white bars represent the NEW stimulus. (a) Ratings of valence were consistently negative indicating that stimuli reinforced during training were rated more negatively after training compared to stimuli not associated with the shock. (**b**) Arousal ratings indicate that CS+ was consistently valued as exciting again compared to CS-. Therefore, both valence and arousal ratings indicate that CS+ acquired aversive properties independently from event timing.

For all analysis, a minimum cluster size of 5 voxels was required. The statistical threshold was set at $p < 0.05$ after the family wise error (FWE) correction. However, in order to reveal activation in the insula and in the striatum, I set the statistical threshold at $p < 0.001$ (uncorrected) and at $p < 0.002$ (uncorrected) to reveal activation in the PCC.

The analysis for the subjective ratings was conducted with between-subjects factor Conditioning (forward, backward) and with within-subjects factors Stimulus (CS+, CS-,

NEW) and Time (before, after conditioning). The awareness of the association between visual stimuli and the electric shock was again conducted with between-subjects factor Conditioning (forward, backward) and within-subjects factor Stimulus (CS-, CS+) and Time (after conditioning, after extinction). All subjective data were analyzed using SPSS for Windows (Version 17.0, SPSS Inc.).

The α level was set at .05 for all statistical tests. Greenhouse-Geisser corrections (GG-ϵ) were used for main effects and interactions involving factors with more than two levels.

### 3.2. Results

#### *3.2.1 Subjective Ratings*

Subjective ratings for the valence indicated a main effect for the time ($F(1, 26) = 16.45$, $p = .000$) as well as a significant Interaction Stimulus x Time ($F(2, 52) = 11.92$, $p = .000$, GG-ϵ = 0.79). Follow up tests indicated that the visual stimuli changed their valence during the experiment. Initially, the valence of the stimuli was rated as 'emotionally' neutral. However, CS+ acquire a negative valence compared to its initial valence after both forward ($t(13) = 2.52$, $p = .026$) and backward ($t(13) = 2.62$, $p = .021$) conditioning. CS+ valence did not differ significant from NEW valence (forward: $t(13) = -0.51$, $p = .618$; backward: $t(13) = -0.44$, $p = .664$), but from CS- valence (forward: $t(13) = -2.42$, $p = .031$; backward: $t(13) = -3.23$, $p = .007$) (Figure 3.1a).

Similarly, arousal ratings indicates a significant main effect for the time ($F(1, 26) = 29.62$, $p = .000$) and a significant Interaction Stimulus x Time ($F(2, 52) = 19.4$, $p = .000$) suggesting that the shapes changed their arousal during the experiment. Specifically, stimulus arousal was rated as neutral at the beginning, but then CS+ acquired higher arousal compared to its initial rating after both forward ($t(13) = -4.12$, $p = .001$) and backward ($t(13) = -4.01$, $p = .001$) conditioning. After the training phase, CS+ was rated as more arousing compared to the NEW stimulus (forward: $t(13) = 2.75$, $p = .017$; backward: $t(13) = 2.16$, $p = .050$), but also compared to the CS- (forward: $t(13) = 4.27$, $p = .001$; backward: $t(13) = 3.86$, $p = .002$) (Figure 3.1b).

No significant effects were found for conditioning in regard to the valence ($F(2, 52) = 0.54$, $p = .589$) and the arousal ($F(2, 52) = 0.82$, $p = .424$; GG-ϵ = 0.81). This suggests that participants experienced the stimulus associated with an aversive event as negative and arousing independently from their timing.

### *3.2.2 Functional Neuroimaging*

Since there is no evidence in humans about backward conditioning, I have first looked into the neural correlates of the two experimental groups separately. Moreover, the first 15 trials of the extinction were analyzed to prevent the within-trial response habituation of amygdala activity (Büchel, et al., 1998; Schiller, Levy, Niv, LeDoux, & Phelps, 2008). Secondly, the forward and the backward conditioning group were compared.

The right *amygdala* (peak voxel: 32, 0, -20, $Z = 3.53$, $p = .015$) and the left *insula* (peak voxel: -34, 16, -16, $Z = 3.35$, $p < .001$) were greatly activated in the presence of forward CS+ referred to forward CS- (see Table 3.1). These activations of the hemodynamic response indicate that the fear conditioning paradigm I applied was appropriate, indeed the neural

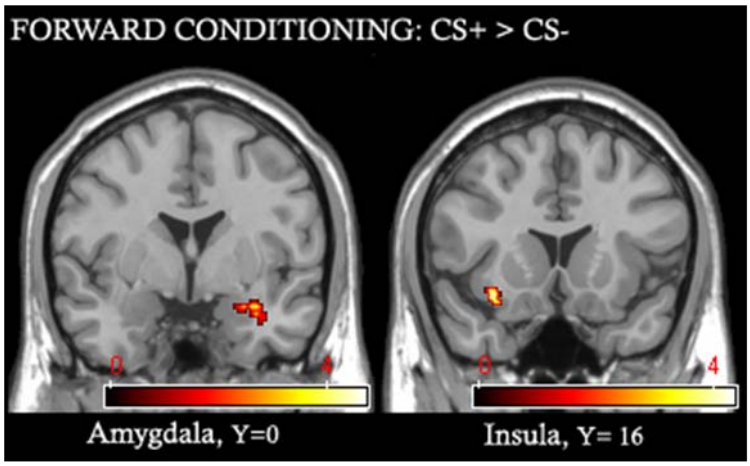



*Figure 3.2.* Neural activation induced by visual stimulus associated with the electric shock after forward conditioning.

Right amygdala (MNI coordinates; peak voxel: 32, 0, -20) and left insula (MNI coordinates; peak voxel: -34, 16, -16) showed greater activation in the presence of the CS+ referred to CS- during the first 15 trials of the extinction. Thus, when a stimulus preceded an aversive event during the training phase, then it activates those brain areas implicated in the processing of aversive events.

correlates of the aversive motivational system were activated (Figure 3.2.).

In regard to the backward conditioning, the left *insula* (peak voxel: -36, 4, 4, $Z = 3.62$, $p < .001$) was strongly activated in the presence of the backward CS+ referred to CS-. And interestingly, the hemodynamic response to backward CS+ was also greater in the right *PCC* (peak voxel: 12, -42, 14, $Z = 3.32$, $p < .001$) and in the right *striatum* (peak voxel: 28, 14, 2, $Z = 4.09$, $p < .001$) (see Table 1 and Figure 3.3). Moreover, contrasts between backward CS+ and the NEW stimulus revealed a great activation in the right striatum (peak voxel: 24, 14, 4, $Z = 3.70$, $p < .001$). Striatum, in particular its ventral part, is involved in reward prediction

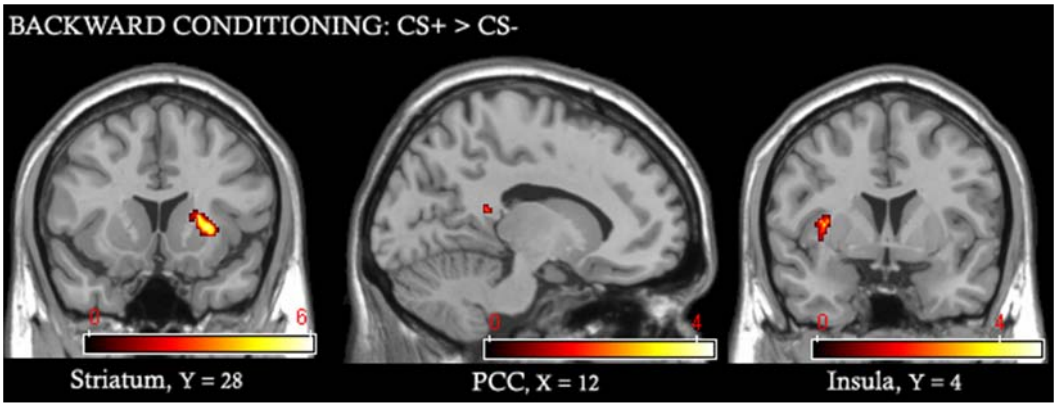



*Figure 3.3.* Neural activation induced by visual stimulus associated with the electric shock after backward conditioning.

The right striatum (MNI coordinates; peak voxel: 28, 14, 2), left insula (MNI coordinates; peak voxel: -36, 4, 4) and right posterior cingulate cortex (MNI coordinates; peak voxel: 12, -42, 14) were more greatly activated in the presence of CS+ than CS- during the first trials of the extinction. Thus, the associated stimulus that followed the shock during the training phase, then acquired appetitive properties and induced activation of the reward brain networks

(Cardinal, et al., 2002), whereas PCC is particularly involved in response to rewarding stimuli (Maddock, 1999). Hence, backward associations with an aversive event involve appetitive cortical networks and might have opponent properties than forward associations. To answer this hypothesis, I contrasted the hemodynamic responses to forward conditioning with that to backward conditioning.

The right *amygdala* (peak voxel: 32, 0, -30, $Z = 3.49$, $p = .016$) was more greatly activated in the presence of the forward CS+ compared to in the backward CS+ both referred to the control stimulus (CS+ > NEW) (see Table 3.2. and Figure 3.4.) indicating that these regions

**Table 3.1**. Activations after either forward or backward conditioning.

| ROI | contrast | *x* | *y* | *z* | *Z* value | *P* value |
|---|---|---|---|---|---|---|
| Forward Conditioning | | | | | | |
| right Amygdala | CS+ > CS- | 32 | 0 | -20 | 3.53 | .015 |
| left Insula | CS+ > CS- | -34 | 16 | -16 | 3.35 | .000 |
| Backward Conditioning | | | | | | |
| left Insula | CS+ > CS- | -36 | 4 | 4 | 3.62 | .000 |
| right Striatum | CS+ > CS- | 28 | 14 | 2 | 4.09 | .000 |
| right PCC | CS+ > CS- | 12 | -42 | 14 | 3.32 | .000 |

The coordinates of the voxel refer to the Montreal Neurological Institute (MNI).

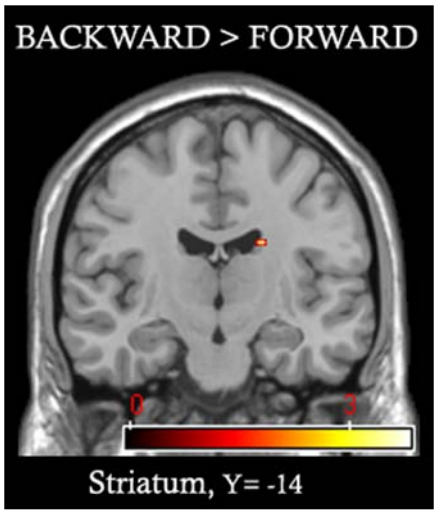



*Figure 3.4.* Differential activation after event learning.

Right striatum activation (MNI coordinates, peak voxel: 20, -14, 24) was greater in the presence of backward CS+ compared to with forward CS+ both referred to the CS- suggesting that backward conditioning involved brain regions that are implicated in reward processing.

were more greatly implicated in forward associations than in backward ones. Therefore, forward CS+ became a signal of the shock according to previous evidence showing the implication of amygdala in the anticipation of aversive events (Dunsmoor, et al., 2007; Ploghaus, Becerra, Borras, & Borsook, 2003; O´Doherty, et al., 2003).

The right *striatum*, particularly in the nucleus caudate, (peak voxel: 20, -14, 24, $Z = 3.35$, $p < .001$) was greatly activated by backward CS+ compared to forward CS+ both referred to CS- (CS+ > CS-) indicating that this region was more greatly implicated in backward association between a neutral visual stimulus and an aversive US (see Table 2). Therefore, backward conditioning greatly involves structure involved in reward processing (Delgado, 2007). However, this activation does not completely

exclude the role of the striatum in learning the contingency awareness (Kluchen, et al., 2009b).

**Table 3.2**. Comparison between forward and backward conditioning.

| ROI | contrast | *x* | *y* | *z* | *Z* value | *P* value |
|---|---|---|---|---|---|---|
| Backward Conditioning | | | | | | |
| right dorsal Striatum | CS+ > CS- | 20 | -14 | 24 | 3.35 | .000 |

The coordinates of the voxel refer to the Montreal Neurological Institute (MNI).

*3.2.3 Contingency Awareness of CS-US Association*

Analysis of the awareness about the association between the visual stimuli and the electric shock indicated that participants of both groups were able to verbalize the association between CS+ and US. Thus, the main effect stimulus ($F(1, 26) = 97.15$, $p = .000$) and time ($F(1, 26) = 25.73$, $p = .000$) were found to be significant. Moreover, the Stimulus x Time ($F(1, 26) = 26.84$, $p = .000$) Interaction resulted significant. And follow up tests indicate that the probability of the electric shock in the presence of CS+ was rated significantly higher than for CS- after both forward conditioning ($t(13) = -19.82$, $p = .000$) and backward conditioning ($t(13) = -5.92$, $p = .000$). However, participant contingency awareness significant decreased after extinction and participants rated the association between CS+ and US with less probability compared to the ratings just after the conditioning phase (forward: $t(13) = 3.39$, $p = .005$; backward: $t(13) = 4.19$, $p = .001$). However, participants still rated the association between forward CS+ and US significantly higher than the association between CS- and US ($t(13) = -4.60$, $p = .000$) and tendent significantly after backward extinction ($t(13) = -1.93$, $p = .076$).

## 3.3. Discussion

The main goal of this study was to look into the neural correlates of forward and backward conditioning. To reach this goal, I collected two groups of participants who underwent either forward (CS+ → US) or backward (US → CS+) conditioning. On the one hand, forward CS+ would become a cue signaling the oncoming of a threat and consequently activate the fear networks like the amygdala. On the other hand, backward CS+ would become a cue signaling the termination of a threat and consequently activate the reward networks like the striatum. These hypotheses were supported. That is, after forward conditioning, CS+ increased amygdala activation (Figure 3.2.), but after backward conditioning, CS+ increased striatum activation (Figure 3.4.). Further regions like the insula were also involved in fear processing, while regions like the PCC were involved in 'relief-like' processing

Forward conditioning is a simple model that explains how organisms make associations between events enabling them to prevent both biological salient events and consequently assure their survival. Similarly here, the forward CS+ became a threat signal and induced fear responses i.e. the amygdala was greatly activated. The amygdala has been implicated in fear acquisition and expression (LeDoux, 1995). Even more than this, it is the crucial structure in fear processing (Davis, & Wahlen, 2001). The ACC has been linked to attentional process of emotional experience as well as in evaluation of emotional stimuli (Lane, Fink, Chau, & Dolan, 1997), while the insula seems to integrate inputs from somatosensory circuitry with the

amygdala's inputs (Phelps, et al., 2001). Moreover, Büchel and colleagues (1998) suggested that the ACC together with the insula provide a route that integrates memory and sensorial inputs to allow then appropriate responses to stimuli that predict future adversities.

According to the literature, these results are not so striking, because they just corroborate previous findings. However, these results are crucial here for two main reasons. Firstly, the classical fear conditioning paradigm was successful in shaping associations between a painful event and a neutral one. The fear conditioning paradigm was thereby established in our institute using the fMRI. Secondly and most important, since forward conditioning is the paradigm par excellence in studying fear and anxiety (Büchel, et al., 1998; Phelps, Delgado, Nearine, & LeDoux, 2004) and the cortical structures as well as the conditioned responses are well known (Bechara, et al., 1995; Lipp, et al., 1994), a working forward conditioning paradigm allows us to have a reference paradigm for the backward conditioning, which is not as established in human research as the forward conditioning.

Participants showed opponent conditioned response after backward conditioning referred to forward conditioning (Table 1 and Figure 3.4.). Namely, cortical regions that are typically involved in reward processes were activated by the backward CS+ suggesting that the backward CS+ acquired a hedonic property which is opponent to that acquired by the forward CS+. If the amygdala is the crucial structure for fear, the striatum seems to be the crucial structure for reward (Delgado, Locke, Stenger, & Fiez, 2003; Delgado, 2007; Knutson, et al., 2001b; O´Doherty, et al., 2006; O´Doherty, et al., 2004; Schultz, et al., 1997; Seymour, Daw, Dayan, Singer, & Dolan, 2007). Knutson and colleagues (2001b), using the monetary incentive delay (MID) task, found a strong activation of the NAcc in humans anticipating rewards but not punishment. Delgado et al. (2003) and O´Doherty et al. (2004) highlighted a specific and dissociable role of the ventral and dorsal striatum for rewarding stimuli. Thus, Delgado et al. (2003) reported that the ventral striatum specifically responds to reward anticipation, whereas the dorsal striatum responded to stimulus salience regardless of its valence. Curiously, O´Doherty and colleagues (2004) even found this dissociation between the dorsal and ventral striatum when using a different paradigm. In other words, these authors used an classical appetitive conditioning paradigm (underlying stimulus-reward association) and another paradigm in which the computer selects between a reward and a neutral stimulus and the participants had to indicate which stimulus has been chosen by the computer (underlying stimulus-response-reward association). The ventral striatum was greatly and specifically activated by the appetitive valence of the stimulus (stimulus-reward association), whereas the dorsal striatum mediates the motor and cognitive responses to a rewarding stimulus (stimulus-response-reward association). The striatum was not the only region to be

strongly activated by backward CS+ presence, but the posterior cingulate cortex (PCC) also showed greater activation in the presence of backward CS+ than with the conditioned stimulus not associated with the shock (CS-). At first glance this activation was somehow surprising. However, there are several findings connecting PCC with action-reward associations (Tabuchi, Furusawa, Hori, Umeno, Ono, & Nishijo, 2005). Measuring the neuronal activity from the PCC of rat's brain, Tabuchi and colleagues (2005) found that the neurons in this region mediated the motor responses to conditioned stimuli but specifically to stimuli associated with rewards. Moreover, McCoy and colleagues (McCoy, Crowley, Haghighian, Dean, &, Platt, 2003) conducted two really interesting studies and pointed out the sensitivity of PCC neurons to reward contingency. In the first study, the authors recorded the activity of single neurons in the PCC of macaques while they shifted their gaze to visual targets for liquid reward and the reward varied in size or probability. Interestingly, the activity of the neurons was modulated by reward size. Noteworthy, McCoy and colleagues (2005) extended these results later, specifying the role of PCC in perceiving the subjective reward size rather than the objective target value. Further supports to these results comes from the Dorris and Glimcher (2004) study in which it appeared that monkey's choices were guided by subjective desirability of a rewarding stimulus and such desirability correlated with the firing of the PCC neurons. Moreover, correlation between PCC activation and the speed of attentional shifts was found by Mohanty, Gitelman, Small, and Mesulam, (2008). In fact, both PCC and attentional shifts were sensitive to the motivational state (hungry/satiety) and to the motivational value of the target (i.e. food items during hunger).

Comparison between forward and backward conditioning revealed great activation in the (dorsal) striatum activation after backward conditioning (Table 2 and Figure 3.6.). Once again conforming the idea that CS+ acquired appetitive properties after backward (O´Doherty, et al., 2004).

These results seem to conform to the opponent-process theory (Solomon, 1980) of acquired motivation. Accordingly, a first process is activated by a stimulus and has similar properties as the stimulation. Afterwards, when the stimulus is over, a second process is initiated and is characterized by opponent properties to the stimulus. Here it then is possible to suppose that the electric shock activated a first aversive process followed by an appetitive after-process. The backward conditioned stimulus might have been associated with such a positive after-process, hence the activation of the appetitive neural networks. However, these results do not also exclude an explanation in terms of the inhibitory theory of Koronoski (1959). In fact according to Koronoski's theory, the absence of an aversive event is treated by the organism as an appetitive event i.e. appetitive processes are initiated. Comparably, the

absence of the aversive shock signaled by the backward CS+ induced appetitive responses such as the striatum activation. Consistently, pain and pleasure seem to share neural circuitry like the amygdala, OFC and NAcc. However, the processes underlying pain and reward operate in an opponent manner inside these regions (for a review, see Lenknes, & Tracey, 2008). Seymour and colleagues (2005) demonstrated that a stimulus associated with the termination of a painful event activated those regions that are specific to reward processing like VTA and the striatum. Despite the authors using a completely different paradigm than mine, the results of both studies overlap and pretty well fit the idea that processes behind the termination of pain (relief) have reward-like effects. Curously, after both forward and backward conditioning, I found a strong activation in the insula. The insula is a region which has been implicated in linking emotions to cognitive processes and behavioral responses (Büchel, et al., 1998; Nitschke, Sarinopoulos, Mackiewicz, Schaefer, & Davidson, 2006; Paulus, & Stein, 2006).

Interestingly, subjective ratings (Figure 3.1.) dissociated from the physiological respond exactly as in the startle study (see Chapter 2). Namely, participants showed appetitive responses to backward CS+ but rated it as emotionally negative and high arousing. Furthermore, it might be that such a dissociation between the explicit (reports) and the implicit (neuronal) conditioned responses is linked to the activation in the insula. Thus, this region might integrate the information coming from the sensorial (amygdala or striatum) and cognitive (ACC or vmPFC) neural circuits and only then initiate explicit responses

In summary, these results once again highlight that the brain treats the termination of a painful/aversive event as a rewarding event. Importantly, these results support and extend the results of the previous study. Thus, the acquisition of an implicit positive valence (e.g. attenuation of the startle amplitude) after backward conditioning may be supported by the activation of the reward networks. Strikingly, this study gives further evidences of the role of timing in associative learning emphasizing its modulation of the new motivational valence acquired by the conditioned event. Hence, punishment becomes to reward (Tanimoto, et al., 2004). Humans are complex organisms and their reactions to an event are mediated not only by sensory and affective processes but also by cognitive ones. Therefore, dysregulation between these processes might induce abnormal behavior or account for psychiatric disorders (Himadi, 1987; Naqvi, & Bechara, 2009; Paulus, 2007).

## 4. Attentional Processes Underlying Event Learning

Studies of emotional perception suggest that visual cortical processing is specifically facilitated for features indicating motivational relevance (Bradley, Sabatinelli, Lang, Fitzsimmons, King, & Desai, 2003). And network theories of emotional processing emphasize the involvement of motivational systems that drive attention toward stimuli of high biological significance (Keil, Moratti, Sabatelli, Bradley, & Lang, 2005; Öhman, & Mineka, 2001). Consistent with this view, it has been shown that highly arousing emotional visual stimuli are detected faster (Öhman, & Mineka, 2001), elicit autonomic reactions that are associated with attentional processes (Lang, 1995) and facilitate sensory processing in visual brain areas (Bradley, Codispoti, & Lang, 2006; Bradley, et al., 2003; Keil, Bradley, Hauk, Rockstroh, Elbert, & Lang, 2002; Schupp, Junghöfer, Weike, & Hamm, 2003). In other words, emotional events that are either aversive or appetitive drive the attention to ensure an organism's survival by activating the appropriate motivational systems (Lang, 1995). As a consequence of such activation, a cascade of behaviors is initiated to cope with the events. Similarly after associative learning, a CS+ becomes more attention-getting (i.e., salient) because its quick detection allows the organism to promptly prime a response and therefore it increases the survival probability. Hence, the concept of "motivated attention" is of special interest for fear conditioning. Indeed, the "motivated attention" might represent a framework to differentiate between reflexive attentional processes driven by the motivational significance of a stimulus (e.g., threat) and volitional attentional processes driven by expectancy (e.g., signaling the threat). In line with this notion, Hamm and Weike (2005) proposed a two-level account for human fear conditioning: An implicit and explicit process. The implicit fear conditioning process is capable of activating defensive motivational system without a declarative knowledge about the association between CS and US (i.e., without contingency awareness) and mainly regulated by the amygdala. The explicit fear conditioning process is also capable of activating motivational defensive system but it involves the declarative knowledge of the relationships between the stimuli (i.e., with contingency awareness) and is mainly regulated by the hippocampus. Therefore, the detection of the CS allows the organism to foresee a salient event as a threat and consequently "to prime" the (defensive) motivational system with or without the contingency awareness depending on the imminence of the threat.

Imaging studies in humans have shown greater activations for the reinforced visual stimulus (CS+) as compared to the non-reinforced stimulus (CS-) in temporal and occipital cortices (Cheng, et al., 2003). The classical conditioning imaging studies (Büchel, et al., 1998; Knight, et al., 2004; LaBar, et al., 1998) in humans have shown the involvement, in addition

to the amygdala but also of the frontal, temporal, and parietal cortices and especially of the anterior cingulate cortex (ACC). Possibly, ACC activation reflects a neural network that mediates attentional and emotional aspects of the conditioned stimulus. In fact, visual cortex seems to be the cortical region for a quick and early discrimination of affective-motivational stimuli (Bradley, et al., 2006; Keil, et al., 2005; Junghöfer, Bradley, Elbert, & Lang, 2001; Moratti, Keil, & Stolarova, 2004; Pizzagalli, Regard, & Lehmann, 1999). Thus, both positive and negative high arousing stimuli enhance attention allocation and involve cognitive processing and high-order associative cortex. Moreover, affectively arousing stimuli, with respect to less arousing stimuli with similar valence, induce greater activation in the visual cortex (Bradley, et al., 2006). Namely, erotic pictures typically activate the visual cortex more than happy families even though these contents were equivalent in rated affective valence. This difference was even more accentuated for pictures of threat and mutilation compared with angry faces.

Steady-state visual evoked potentials (ssVEP) have often been used to study the electrocortical responses underlying attentional processes (Müller, & Hübner, 2002; Müller, & Hillyard, 2000; Silberstein, Ciorciari, & Pipingas, 1995). The ssVEP is an oscillatory brain response elicited by a flashing visual stimulus presented at a particular driving frequency (Regan, 1989). Power differences between experimental conditions are thought to reflect differential engagement of neural activity in brain regions supporting attentional processes. Müller and Hillayard (2000) reported an increase of ssVEP amplitude in response to attended stimuli compared to unexpected ones. Additionally, ssVEP amplitude has been observed to be enhanced when viewing flickering picture stimuli rated as being high arousing with most pronounced differences at the central posterior as well as parietotemporal recording sites (Keil, Sabatinelli, Ding, Lang, Ihssen, & Heim, 2009; Keil, et al., 2005; Keil, Gruber, Müller, Moratti, Stolarova, Bradley, & Lang, 2003). Keil and colleagues (2009) reported enhanced ssVEP amplitude and accelerated relative timing (phase) of ssVEP by the presentation of high arousing stimuli compared to neutral ones. In agreement with this, Moratti and colleagues (2004) found that the steady-state visual evoked field amplitude (ssVEF; the magnetocortical counterpart of the ssVEP) was greater for high arousing stimuli than for low arousing ones, beside they found a predominance of the right hemisphere. In another study, Gray and coworker (Gray, Kemp, Silbertein, & Nathan, 2003) investigated cortical oscillatory activity during the anticipation of an electric shock and found a greater involvement of frontal, temporal and occipital electrode sites. Intriguingly, such an increase of the ssVEP amplitude

was more pronounced during the anticipation of an imminent threat or danger that is typically associated with sympathetic arousal and fight or flight reactions.

Currently there is a strong debate regarding the role of contingency awareness in fear conditioning as a crucial factor for fear acquisition or not (Devriese, et al., 2004). The contingency awareness is the capacity of the participants to verbalize the association between a conditioned and an unconditioned stimulus, it is then possible to presume that explicit attentional processes are required for such a verbalization. Participant contingency awareness seems to modulate some physiological fear responses after conditioning like heart rate (HR) (Redondo, & Marcos, 2003). Beside the growing literature regarding the importance of contingency awareness in fear conditioning (Kluchen, Kagerer, Schweckendiek, Tabbert, Vaitl, & Stark, 2009b; Kluchen, et al., 2009a; Tabbert, Stark, Kirsch, & Vaitl, 2005), there are only few studies combing fear conditioning and attentional processes (Moratti, et al., 2006; Moratti, & Keil, 2005). Moratti and colleagues (2006) found that only participants showing an increased heart rate (HR), i.e. expressing fear, also differed in their cortical responses. Only those participants who showed a HR acceleration by CS+ was the ssVEF amplitude for the stimulus associated with the aversive US (CS+) enhanced compared to the stimulus not associated with US (CS-).

So far it is clear that fear responses to CS+ are increased after forward conditioning, but fear responses are inhibited after backward conditioning and appetitive reaction to backward CS+ may also occur. As indicated by past literature, negative stimuli as high arousing negative pictures and fear conditioned stimuli catch more attentional resources. Although appetitive high arousing stimuli may prompt extensive activity in the visual cortex and catch attentional resources in a way similar to negative high arousing stimuli (Bradley, et al., 2006, Moratti, et al., 2004). Unfortunately appetitive stimuli are considered even less in the emotional/attentional research field, despite their importance for survival. Moreover, the role of attentional processes in event learning has been relatively neglected.

Based on this evidence, the hypotheses were that both forward and backward CS+ associated with an aversive US would induce greater ssVEP amplitude compared to a control (NEW) stimulus because both stimuli are affectively more salient and catch more attentional resources. However, forward CS+ would induce higher ssVEP amplitude compared to the backward CS+.

## 4.1. Method

### *4.1.1. Participants*

Thirty-seven university students participated voluntarily in the study. Participants were randomly assigned to either the forward or the backward conditioning group. During the forward conditioning, the US was delivered *after* the CS+; whereas during the backward conditioning group, the US was delivered *before* the CS+. Three participants were excluded from the analysis because of noise-contaminated data. Overall, thirty-four participants were included in the analysis (mean age = 22.76 years; SD = 2.5; range from 19 to 30 years old) 17 of which were female. Each group consisted of 17 participants. All participants were free of neurological, psychiatric or chronic pain diseases.

Participants were right-handed (as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971), and they reported a mean state anxiety score of 34.1 (±5.7) and a mean trait anxiety score of 39.4 (±7.4).

All participants were paid 12 Euro for participating in the experiment.

### *4.1.2. Stimulus Material*

The US consisted of a single unipolar electric shock of 200 ms duration delivered via two surface 7 mm AgAgCl electrodes which were attached to the left forearm with a spacing of 15 mm (Skrandies, & Jedynak, 2000). The electric pain threshold was computed for every participant before the acquisition phase by means of a LabVIEW 5.1 (National Instruments Product) *ad hoc* program which administered increasing electric stimuli. Electric stimuli were delivered by a battery powered constant current stimulator controlled by PC through the parallel port. The minimal stimulus had 39 μA intensity, but stimulus intensity was randomly increased until the painful threshold was reached. Maximum current level which could be delivered was 10 mA. Subjects had to evaluate every electric pulse using a 10-point VAS representing different levels of pain intensities, where 0 meant “no pain at all” and 10 meant “unbearable pain”. The program computed an on-line regression coefficient of this subjective evaluation for precisely determining pain thresholds. The value corresponding to the pain threshold was reached and computed when the average pain perceived in three consecutive electric pulses surpassed the pain threshold corresponding to the critical level of 5. After this phase, in the following session the intensity current was increased by 30% with respect to individual pain threshold and was used as painful US during the acquisition phase.

The stimulus material consisted in three simple geometrical shapes (a square, a circle and a triangle), gray-shaded gratings oriented perpendicularly. The shapes had the same area of 48.8

$cm^2$. The luminance modulation was achieved by showing the stimulus for 40 ms followed by a 40 ms black screen. This resulted in an 80 ms on/off cycle. The flashing stimuli were shown for 8000 ms by presenting 100 of the 80 ms on/off cycles (for reference see Moratti, Clementz, Gao, Ortiz, & Keil, 2007). One visual stimulus was always associated with the US (CS+), while another was never associated with the US (CS-) and the third shape was presented only during the test phase (i.e., the extinction) and served as control stimulus (NEW stimulus). Stimuli were counterbalanced across participants

Participants were asked to fill in the Italian version of the Spielberger's STAI (Pedrabissi, & Santinello, 1989). The STAI is a well-known psychometrical instrument consisting of two 20 item scales assessing state and trait anxiety. The items are rated on 4-point Likert-type scales from "almost never" to "almost always" and ask the participants to describe how they feel either in that moment (STAI-state) or generally (STAI-trait).

### *4.1.3. Procedure*

After having signed the informed consent form, participants were seated in a comfortable chair in a sound attenuated room next to the experimenter's room. Participants filled out the questionnaires: Edinburgh Handedness Inventory and both the state- and trait-STAI. Pain threshold was assessed after electrode attachment as described above. Before starting, participants were told that a series of electric shock and of geometrical shapes would be presented on the screen and that they should keep these pictures in their visual focus. No clues were given regarding the contingency between the stimuli.

The experiment consisted in two phases alternated by subjective rating sessions.

During the *conditioning* (i.e., acquisition phase), two of the three geometrical shapes were presented 16 times each, i.e. it consisted of 32 trials. The visual stimuli were presented on the black background of a 19'' computer screen for 8 s durations. The screen was located 140 cm from the participants' eyes. For the forward conditioning group, US followed CS+ and was presented at the offset of CS+ (ISI = 8 s). For the backward conditioning group, US preceded CS+ and was presented 6 s before CS+ onset (ISI = -6 s). Both forward and backward CSs+ were always associated with the US (contingency 100%). The ITI lasted randomly from 20 s to 30 s (mean of 25 s). The presentation order of the visual stimuli was randomized with the only restriction being that the same stimulus was to be repeated no more than twice in a row.

During the *extinction* (i.e., test phase), the two conditioned visual stimuli (CS+ and CS-) were presented again but with a new additional neutral shape (NEW stimulus). Each stimulus was presented 12 times so that extinction consisted of 36 trials. The stimuli were presented randomly with the only restriction being that the same stimulus was not presented more than

twice in a row. Again, visual stimuli lasted 8 s on the black computer screen and the ITI was 20-30 s in duration (mean of 25 s).

Before and after conditioning, participants had to rate the valence and the arousal of the geometrical shapes (i.e., *subjective rating* phase). A visual analogical scale (VAS) ranging from 1 until 9 (Bechara, et al., 1995) was presented on the computer screen. 1 meant "very unpleasant" for the valence and "calm" for the arousal; while 9 meant "very pleasant" and "exciting" respectively. Participants made evaluations by pushing a button on a keyboard corresponding to their rating.

An additional scale was presented after both conditioning and extinction and it served to measure the contingency awareness of the participants regarding the association between CS+ and US. This VAS ranged from 0 to 100 in which 0 indicated no association between CS+ and US, whereas 100 indicated association between CS+ and US (Knight, et al., 2003). Also in this case, participants rated the association by pushing a button on the keyboard in front of them.

### *4.1.4. Physiological Recording and Data Reduction*

Electrophysiological activity was continuously recorded in the DC mode by 39 electrodes, 32 placed on an elastic cap (Electrocap) according to the International 10-20 System and the other 7 applied below and upon the left eye (VEOG), on the external cathi (HEOG), on the nasion (Nz) and on the mastoids (A1, A2). All cortical sites were online referred to Cz. Data were recorded using the software Acquired NeuroScan 4.1 version. Bandwidth ranged from DC to 100 Hz (6 dB/octave). Sampling rate was set at 500 Hz, low-pass filter at 150 Hz and the impedance was kept below 5 kΩ.

Offline analyses of the physiological data were conducted using Analyzer 2.0 (BrainProducts Inc.). Data were offline low-pass filtered at a frequency of 30 Hz (24 dB/octave) and high-pass filtered at a frequency of 1 Hz (24 dB/octave). Eye movements and blinks were observed offline by visually inspecting the vertical electro-oculogram and afterwards average reference was applied. Epochs included 1000 ms pre-stimulus and 9000 ms post-stimulus for each condition that is CS+, CS- and NEW stimulus. Moreover, conditioning and extinction were analyzed separately for each experimental group. A 200 ms pre-stimulus segment was subtracted and served as a baseline. Each epoch containing electro-myogram artifacts or blinks exceeding 75 μV was excluded from further analysis. The mean rejection rate was 25% of trials. Based on these criteria, three participants were not considered in the analysis. The averaged epochs were submitted to a Fast Fourier Transformation analysis (FFT) to estimate the evoked spectral power within the frequency range of 11-13 Hz

that includes the driving frequency of 12 Hz (Keil, et al., 2003; et al., 2007; Müller, & Hillyard, 2000). Thereafter, a complex demodulation at the stimulation frequency of 12 Hz was applied in order to obtain stimulus-driven amplitudes. The process of complex demodulation extracts the modulating signal from the carrier frequency, in this case the stimulation frequency of 12 Hz (Regan, 1989). Firstly, the complex demodulation was applied to the eight second stimulus duration in order to look at the general attentional processes driven by the conditioned stimuli. The complex demodulation was performed on the averaged epochs of CS+, CS- and the NEW stimulus for each experimental session (conditioning and extinction) and also for each experimental group (forward and backward conditioning group) separately. Secondly, the eight-second epochs were divided into four temporal windows of two seconds each to study evolution of attentional processes across the time (Moratti, et al., 2006).

### *4.1.5. Data Analysis*

The mean 12 Hz power values of CS+, CS- and NEW were estimated separately for the acquisition and the extinction, for forward and backward conditioning. And the electrodes were clustered in *left posterior* (left: P3, O1), *central posterior* (middle: Pz, Oz) and *right posterior* (right: P4, O2). Firstly, ANOVAs for the ssVEPs included as between-subjects factor conditioning (forward conditioning, backward conditioning) and as within-subjects factors both stimulus (CS-, CS+, NEW) and site (left, middle, right). Secondly, stimulus presentation was divided in four temporal windows corresponding to the first 2 s (t1), 2-4 s (t2), 4-6 s (t3) and 6-8 s (t4) after stimulus onset. Hence, the following analysis of the ssVEPs had the same factorial structure 2 x 3 x 3, but with an additional within-subjects factor time (t1, t2, t3, t4).

The subjective ratings for the valence and arousal were analyzed with repeated measures ANOVA containing as between-subjects factor conditioning (forward conditioning, backward conditioning) and as within-subjects factors both the stimulus (CS+, CS-, NEW) and the time (pre-, post-conditioning).

As final step, the ANOVAs for the contingency awareness ratings included a between-subjects factor the conditioning (forward conditioning, backward conditioning) and as within-subjects factors both the stimulus (CS+, CS-) and the time (post-conditioning, post-extinction).

The alpha level was set to .05 for all statistical tests and when necessary, the Greenhouse-Geisser correction (GG-ε) was applied.

Statistical analyses were conducted by using the Software SPSS for Windows (Version 17.0, SPSS Inc.).

## 4.2. Results

### *4.1.1. Subjective Ratings*

The ANOVA did not show any significant interaction between stimulus and conditioning for both valence and arousal ratings (valence: $F(2, 64) = 1.23$, $p = .298$; arousal: $F(2, 64) = 0.72$, $p = .490$) indicating that the conditioning type did not modulate the subjective ratings

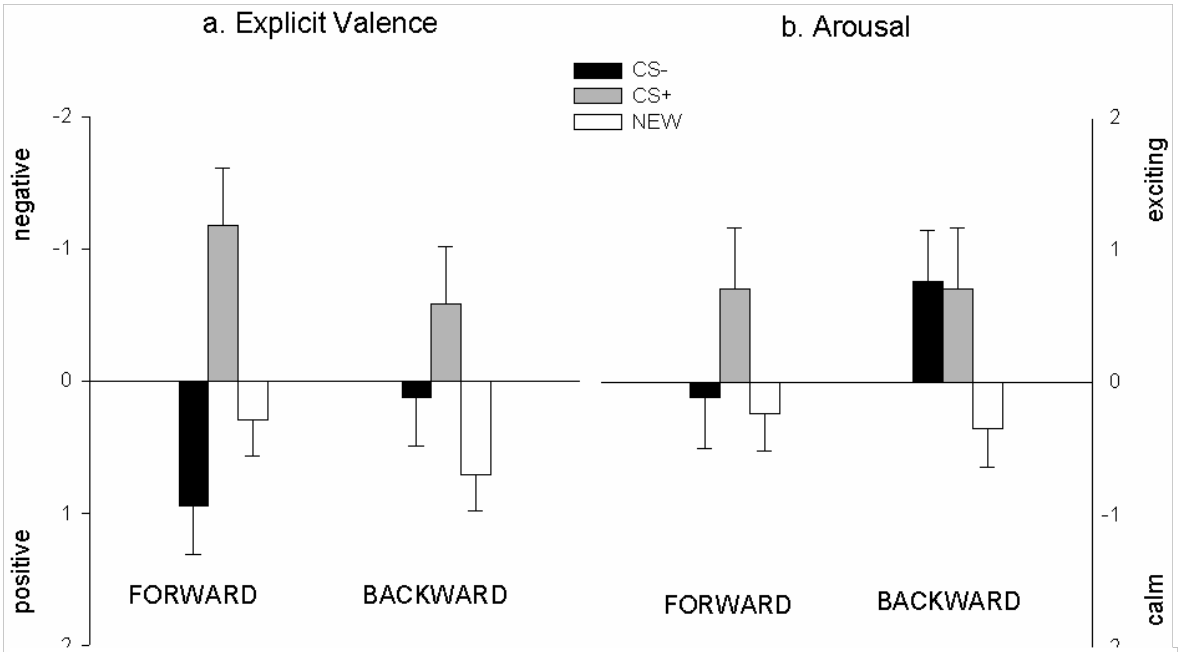



*Figure 4.1.* Explicit valence and arousal assessed with subjective ratings after forward and backward conditioning.

Bars (with standard error) represent the ratings for the visual stimuli after forward and backward conditioning. Black bars correspond to CS-; gray bars to CS+ and white bars to NEW stimulus. Participants rated valence and arousal of the visual stimuli using a visual analogical scale (VAS) from 1 (negative, calm) until 9 (positive, exciting) in which 5 meant a neutral rating (neither negative nor positive for the valence; neither calm nor exciting for the arousal). Values on the y-axis represent difference values between participant ratings and the neutral (i.e., 5). **a.** Ratings of the valence were consistently negative for CS+ after the acquisition phase indicating that stimuli reinforced during conditioning (CS+) were rated more negative compared to the neutral control stimulus (NEW). **b.** Ratings of arousal were not significantly modulated by event timing.

differently. However, ANOVAs showed a main effect of stimulus for the valence ($F(2, 64) = 6.86$, $p = .002$) indicating that CS+ was rated as more unpleasant than the CS- and NEW stimuli. But the arousal ratings showed a tendency to significance for the main effect stimulus ($F(2, 64) = 3.01$, $p = .066$, GG-$\varepsilon = 0.84$). No significant main effect of time was found (valence: $F(1, 32) = 0.00$, $p = 1.0$; arousal: $F(1, 32) = 0.55$, $p = .464$). However, the interaction of Stimulus x Time was found to be significant for the valence ($F(2, 64) = 6.25$, $p = .003$) Follow-up tests indicated that the valence did not differ among the three pictures before conditioning and all shapes were rated as "emotionally" neutral. After conditioning, CS+ valence significantly differed from NEW valence (forward: $t(16) = -2.55$, $p = .021$; backward: $t(16) = -2.52$, $p = .023$ – Figure 4.1a). In other words, CS+ was rated with more negative valence compared to the NEW stimulus. Moreover, forward CS+ valence was rated more negative compared to its initial valence ($t(16) = 2.63$, $p = .018$). As expected, valence of the NEW stimulus remained neutral during the whole experiment.

No significant interaction was found for the arousal ($F(2, 64) = 1.48$, $p = .236$).

### *4.1.2. Contingency Awareness of CS-US Association*

Regarding the knowledge about the association between CS+ and US, ANOVA showed a main effect stimulus ($F(1, 32) = 37.71$, $p = .000$) indicating that participants correctly learned the association between CS+ and US. Thus, participants of the forward conditioning group reported association between US and CS+ and no association between US and CS- ($t(16) = -7.81$, $p = .000$); while participants of the backward conditioning group also reported such an association but with a tendency to significance ($t(16) = -2.09$, $p = .053$). Such an awareness was maintained after the extinction (forward: $t(16) = -5.17$, $p = .000$; backward: $t(16) = -2.18$, $p = .045$).

Interestingly, the interaction between stimuli and conditioning ($F(1, 32) = 7.38$, $p = .011$) was significant. Thus, the contingency awareness of the participants after forward conditioning was significantly higher compared to the contingency awareness of the participants who underwent the backward conditioning ($t(32) = 2.29$, $p = .029$).

Since the contingency awareness has a crucial role on the modulation of conditioned responses and on attentional processes, the possible modulation of the contingency awareness on the ssVEP amplitude was also considered into the analyses. In other words, the two experimental groups were split in aware and unaware participants according to their median. Aware participants were those who rated the association between CS+ and US higher than the median value and unaware participants fell below the median value.

*4.1.3. Steady-State Visual Evoked Potentials*

Repeated measures ANOVA indicated a significant Stimulus x Conditioning interaction ($F(1, 32) = 5.85$, $p = .021$) during the acquisition phase indicating that the CS+ induced an overall higher ssVEP amplitude than CS-. Analyses of the ssVEPs amplitude during the extinction did not show any significant effect.

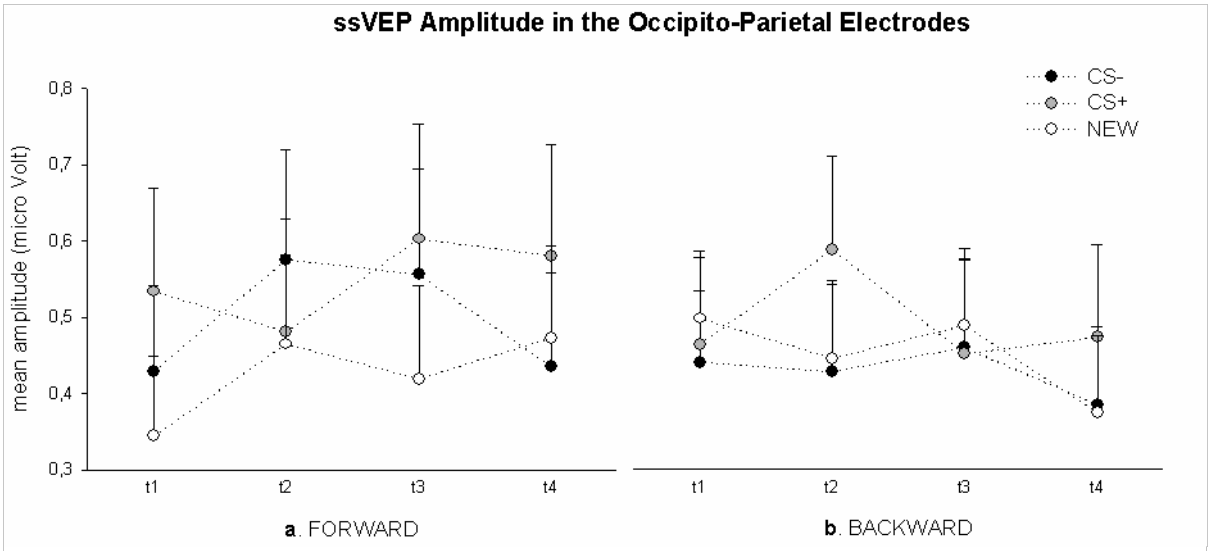



*Figure 4.2.* Visual cortex activation during stimulus presentation after the acquisition.

The values on the y-axis indicate the mean amplitude of the ssVEPs. On the x-axis, the temporal windows of two seconds are depicted. t1 represents the first two seconds after stimulus presentation, t2: 2-4 s, t3: 4-6 s and t4: 6-8 s. Dots (with standard error) indicate the mean ssVEP amplitude during CS- (black dots), CS+ (grey dots) and the NEW stimulus (white dots). **a**. After forward conditioning, participants showed enhanced ssVEP amplitude during the last 4 s (t3, t4) of the CS+ presentation, i.e. *just before* the aversive US. **b**. After backward conditioning, participants showed enhances ssVEP amplitude during the first 4 s (t2) of the CS+ presentation, i.e. '*just' after* the aversive US.

According to Moratti's study (2006), those participants who showed HR increase ("conscious" fear response) showed higher ssVEP in the presence of CS+ as well. Moreover, such an increase was induced during the last seconds of the visual stimulus that is just before the US. Based on these evidences, the data were reanalyzed considering the ongoing attentional processes during stimuli. To this purpose, the eight seconds of stimulus presentation were divided into four temporal windows of two seconds each. Repeated measures ANOVA showed a significant Stimulus x Conditioning interaction ($F(1, 32) = 5.83$, $p = .022$) during the acquisition. During the extinction, the Stimulus x Time x Conditioning

interaction resulted significant (F(6, 192) = 3.93, $p$ = .003, GG-ε = 0.74) (Figure 4.2). Follow-up tests did not indicate significant differences between forward and backward conditioning and between the CS+ and the NEW stimulus. Probably, the post hoc was not significant because of the high inter-individual variability. Nevertheless, these results indicate a modulation of ssVEP depending on the US timing.

Moreover, Moratti and colleagues (2006) highlighted that those participants who showed HR increase (i.e., strong and "aware" fear responses), also showed stronger ssVEF modulation. Hence, the forward and the backward group were split into aware and unaware participants according to their median. Repeated measures ANOVA for the acquisition showed a significant Stimulus x Conditioning interaction (F(1, 30) = 7. 37, $p$ = .011). The significant Stimulus x Site interaction (F(2, 60) = 4.05, $p$ = .032; GG-ε = 0.79) suggests higher ssVEP amplitude in the right posterior electrodes in the presence of CS+ compared to

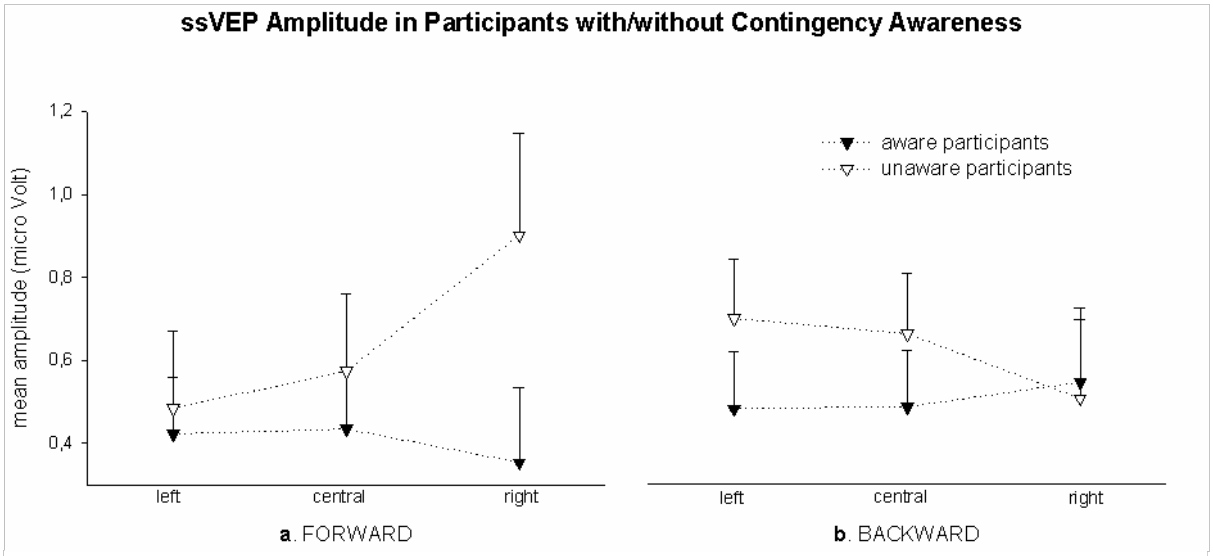



*Figure 4.3.* Visual cortex activation during stimulus presentation after the acquisition in participants with or without contingency awareness.

The values on the y-axis indicate the mean amplitude of the ssVEPs. On the x-axis there are the clusters in the occipito-parietal regions: P3, O1 the left hemisphere, Pz, Oz the central line and P4, O2 the right hemisphere. Participants who correctly verbalized the contingency between US and CS+ are depicted by the black triangles (with standard error), whereas white triangles (with standard error) depict participants who not correctly verbalized the contingency. **a**. After forward conditioning, unaware participants showed enhanced ssVEP amplitude especially in the right sites. **b**. After backward conditioning, there were no particular differences between the two groups.

CS- ($t(33) = -2.15$, $p = .039$), but without differences across time. And the significant Stimulus x Site x Time interaction ($F(6, 180) = 2.99$, $p = .046$; GG-$\varepsilon = 0.41$) suggests high ssVEP amplitude in the right hemisphere in the presence of CS+ compared to CS- especially during the last seconds of the stimulus presentation ($t(33) = -2.14$, $p = .040$), but again without modulation by event timing. Furthermore, the Stimulus x Awareness interactions ($F(1, 30) = 0.06$, $p = .806$) and Stimulus x Conditioning x Awareness interaction ($F(1, 30) = 3.46$, $p = .073$) probably did not reach the significance because the participants did not yet learn the association between the stimuli.

During the test phase (i.e., extinction), a three way interaction resulted significant (Site x Conditioning x Awareness: $F(2, 60) = 7.83$, $p = .007$; GG-$\varepsilon = 0.55$). Thus, ssVEP amplitude showed a tendency to significance with higher values in the right posterior sites in the unaware participants of the forward conditioning group compared to the aware participants ($t(15) = -1.82$, $p = .089$) (Figure 4.3.). Finally, the interaction Stimulus x Time x Conditioning ($F(6, 180) = 4.27$, $p = .002$; GG-$\varepsilon = 0.69$) resulted again significant.

### 4.3. Discussion

The aim of the study was to investigate the attentional processes after both forward and backward conditioning by means of ssVEP amplitude. Using the technique of complex demodulation, I investigated the changes in cortical activation through stimulus presentation finding that a previously biologically irrelevant stimulus acquires motivated properties and salience through the association with an aversive and biologically relevant stimulus. Indeed, participants showed increased ssVEP amplitude in visual and parietal cortex in the presence of both forward and backward CS+. Such an increase was more pronounced during the last seconds of forward CS+, i.e. *just before* the shock, and during the first seconds of backward CS+, i.e. *just after* the shock. Therefore, visual attention seems to be allocated to aversive cues, a strategy probably aimed at optimizing fast and efficient response to threatening events.

The forward conditioning results are consistent with previous studies in which a flickering CS+ associated with an aversive US induced higher ssVEP amplitude in parietal and occipital brain areas (Moratti, et al., 2006; Moratti, & Keil, 2005). This greater amplitude was increased during the late stimulus presentation just before US onset for CS+. In both studies, Moratti states that such an enhancement of ssVEP amplitude is associated to the activation of the fear system. In fact, participants who showed acceleration of the heart rate demonstrated stronger ssVEP increase in visual and parietal cortex during CS+. And according to the two level account for human conditioning, the heart rate acceleration is an indicator of the activation of the fear system (Hamm, & Vaitl, 1996; Hamm, & Weike, 2005). Consistent with

this, in the present study, greater ssVEP amplitude has been found during the last seconds of the forward CS+ (Figure 4.2a). However, such a difference was found only during the extinction but not during the acquisition, probably because participants still had to learn when exactly the US would be delivered. Furthermore, Moratti and colleagues (2006) also examined whether enhanced cortical processing of reinforced stimuli was due to the expectancy of an aversive stimulus (i.e., the contingency awareness) or just to the activation of the fear system (i.e., automatic and autonomic responses) in their study. There is in fact good evidence that the processing of emotional information is fast and independent of conscious awareness (Öhman, 2005; Pessoa, 2005) and the amygdala seems to provide the substrate for the automatic processing of emotional stimuli (Büchel, et al., 1998; Hamm, & Weike, 2005). Moratti suggested that awareness of the association between CS+ and US is a requisite for fear conditioning, but it is not sufficient. Thus, enhanced ssVEP amplitude has been observed in the presence of CS+ in those participants who showed heart rate acceleration, whereas participants who showed heart rate deceleration (a response related to the expectancy) did not enhance ssVEP amplitude by CS+. Accordingly in this study, ssVEP amplitude was enhanced in the presence of CS+ during conditioning but there were no differences between participants who showed contingency awareness and those who did not. Therefore, also in this study, the contingency awareness seems to be a requisite for conditioning but it is not sufficient on its own. Thus, (fear-)attentional responses are increased in the presence of the forward CS+, but after forward conditioning even unaware participants showed greater activation in the right areas in the presence of CS+ (Figure 4.3a). This strong activation of the right hemisphere is not completely surprising, since the right hemispheric visual cortex has been found particularly sensitive to affective arousing stimuli (Bradley, et al., 2003; Keil, et al., 2005; Moratti, et al., 2004).

Although it seems plausible to think in term of activation of fear system by forward CS+, how can the greater ssVEP amplitude induced by the backward CS+ be explained? Thus far, I supported the hypothesis that depending on event timing, an aversive event may induce either aversive or appetitive conditioned responses. In particular, backward associations with an aversive event seem to provoke the activation of the appetitive motivational system that is characterized by appetitive responses. Coherently with the idea that high arousing appetitive stimuli are as attention-catching as the high arousing aversive ones (Bradley, et al., 2003; Keil, et al., 2009), backward CS+ induced here greater attentional allocation than CS-. Moreover, motivated attention seems to be allocated mainly during the first seconds of backward CS+ unlike forward CS+ (Figure 4.2b). Considering the US timing, participants in

the backward conditioning group received the electric shock a few seconds before the backward CS+. Therefore, it seems plausible to think that the first seconds of the backward CS+ are the most important to survival. Indeed, the participants realize at once by viewing the backward CS+ that the painful shock is over and do not need all 8 s of stimulus presentation to realize this. Hence, attentional resources seem to be directed to the localization of the aversive event in order to promptly react and to better assure survival.

However, participants did not report significant changes in the arousal ratings of the conditioned stimuli. Since the arousal boots motivational activation of fear and appetitive systems (Bradley, Codispoti, Cuthbert,& Lang, 2001), the inability to recognize the stimulus associated with the shock as more activating makes more difficult results interpretation. Indeed, as the stimulus increases its salience, attention allocation and ssVEP amplitude also increase. Therefore, if the participants did not experience the CS+ as more arousing, this could have "weakened" the cortical responses or may have induced more variability in the physiological responses among the participant. Regarding the valence, CS+ was rated as "emotionally" negative after both forward and backward conditioning confirming previous results. Hence, both forward and backward associations with an aversive event did lead to negative explicit valence of the events. These results do support dual-process theories (Bechara, 2005; Strack, & Deutsch, 2004) as Study 1 and Study 2 in this thesis do. Indeed, event learning modulated the "implicit" arousal (i.e., ssVEP amplitude) but not the "explicit" arousal (i.e., subjective ratings) of the visual stimuli.

However, it is difficult to state firm conclusions after this experiment because the follow-up tests did not really reach the significance level. Probably this is the consequence of the high variance in the sample and low statistical power. It would be interesting to carry on a similar study with more participants which could highlight larger size effects. On the other hand, I would also presume that the aversive US here used, compared to the other US used in Study 1 and Study 2 was not able to induce higher arousal after the acquisition.

In conclusion, event timing might influence attentional processes toward salient stimuli. Thus, visual attention seems to be allocated to aversive events, a strategy probably aimed at optimizing fast and efficient response to events. Moreover, dual theories are still supported since the implicit and automatic responses (i.e., the cortical activation) showed an opposite pattern depending on the event timing, which did not occur for the explicit and cognitive responses (i.e., the verbal reports). However, these conclusions on the ssVEP amplitude modulation by the event timing deserve further investigation aimed at depending the critical factors playing a role in differentiating backward and forward conditioning.

# 5. General Discussion

Originally, Dickinson (1979), Koronoski (1967) and Solomon (1980) suggested that the affects are driven by two motivational systems: The appetitive system and the defensive system. The former is prototypically expressed by behavioral approaches like consummatory, sexual or nurturant behaviors. The latter is prototypically expressed by avoidance like defensive, protective or withdrawal behavior. In addition, Lang (1995) explicated that these two motivational systems have two dimensions: Valence and arousal. Accordingly, the arousal indicates the activation and the intensity of motivational system activation; whereas the valence indicates which system is active; either the appetitive (i.e., positive valence) or the defensive (i.e., negative valence) system. These motivational affective systems are affected by many other factors like personality, context and culture (Bradley, et al., 2001). However, I am not going to elaborate on these aspects since they are not the focus of this thesis. Lang (1995) said that *emotions are action dispositions* and suggested that emotions are systemic responses occurring when a motivational system is strongly activated and prepare the organism for action. In these terms, emotions are not actions per se but dispositions to act founded on the activation of either the appetitive or defensive motivational system. Depending on the situation, emotions induce appropriate and prompt reactions facilitating the survival of individuals and animals. Importantly, these two motivational systems operate in an opponent fashion that is the activation of one system induces inhibition of the other one and vice versa. Supportively, brain states seem to be organized along two systems as well. To simplify, they are the striatum at the core of the appetitive system (Schultz, et al., 1997) and the amygdala at the core of the defensive system (Davis, 2006; LeDoux, 1995; Öhman, Carlsson, Lundqvist, & Ingvar, 2007).

Classical conditioning may account for emotions (i.e., such *action dispositions*) since it explains how a stimulus acquires a meaning that may then motivate and elicit specific reactions. In a classical conditioning paradigm, a neutral stimulus acquires affective and motivational properties by virtue of being paired with a biologically significant event like a pain or reward (Cox, et al., 2005; Delgado, et al., 2006; Fendt, et al., 1999; Lipp, et al., 1994; O´Doherty, et al., 2004; Phelps, et al., 2001). After just a few pairings the organism reacts to the neutral stimulus (now called conditioned stimulus; CS+) similarly as it does to the unconditioned salient event (US) that is with approach behavior when US was a reward (Cohen, Axmacher, Lenartz, Elger, Sturm, & Schlaemfer, 2009; Cox, et al., 2005; Delgado, 2007; Rolls, 2000) or with avoidance behavior when was pain (Bechara, et al., 1995; Büchel, et al., 1998; LaBar, et al., 1998). The former indicates activation of the appetitive system, the

latter that of the defensive system. Hence, the mechanisms behind classical conditioning allow understanding of how animals and humans react to imminent biologically significant events through the activation of the appropriate motivational system (Lang, 1995). In other words, simple organisms like a fruit fly or complex organisms like a human learn how to predict events that are relevant for their survival and to adapt themselves accordingly by means of the associative learning.

Thus far, I clarified how the presentation of two stimuli in sequence (i.e., CS+ first, followed by a biologically salient event; US) determines those learning processes that allow the organism to anticipate events and to facilitate their survival. However, some evidence showed that not only the aversive or appetitive properties of the US give rise to organism's conditioned responses, but also the time sequence (i.e., timing) of these events seems to play a crucial role. In fact, few animal studies highlighted that a temporal sequence between a CS+ and an US may determine which kind of conditioned reactions to CS+ the animals show, despite the affective properties of US. In other words, rats (Salvy, et al., 2004), mice (Cunningham, et al., 2002) and fruit flies (Tanimoto, et al., 2004) avoided the CS+ (a chamber or odor respectively) when it preceded an aversive event that was wheel running, an intragastric injection of ethanol or an electric shock respectively. Importantly, animals approached or preferred the CS+ when it followed the aversive event during the acquisition phase that is mice and rats developed conditioned place preference (CCP) and fruit flies flew into the chamber presenting the conditioned odor. Therefore, backward association between events (US → CS+) induced approach behaviors indicating that CS+ acquired appetitive properties despite the aversive valence of US. Curiously, there is no evidence concerning the modulation of event timing in humans.

It is thus of interest whether event timing also modulates conditioned responses in an opposing fashion in humans who are capable of cognitions concerning the associations. Specifically, the hypotheses were that a conditioned stimulus after forward conditioning (CS+ → US) would acquire aversive properties and as a consequence the motivational defensive system would be activated. While a conditioned stimulus after backward conditioning (US → CS+) would acquire appetitive properties and as consequence the motivational appetitive system would be activated. The activation of the defensive system can be indicated by the increased amplitude of the startle response and by the activation of the amygdala in response to forward CS+. The activation of the appetitive system can be indicated by the decreased amplitude of the startle response and an activation of the striatum. These hypotheses were supported. Thus, startle responses were potentiated in the presence of forward CS+ and

attenuated in the presence of backward CS+. Moreover, cortical areas that mediate defensive/fear processing were strongly activated in response to forward CS+ while cortical areas that mediate reward processing were greatly activated in response to backward CS+. These results indicate that CS+ acquired aversive properties after forward conditioning but appetitive properties after backward conditioning. These findings confirm the animal studies and conform well to Solomon's (1980) opponent process theory of acquired motivation. This theory assumes that two processes are initiated by the stimulus appearance. Firstly, the *a* process is induced by the stimulation and has similar affective properties as the stimulus, e.g. if the stimulus is aversive, such as an electric shock, the *a* process induces a State A holding aversive characteristics. As soon as the stimulus is over, the *b* process becomes stronger and induces a State B holding the opponent characteristic to that of State A that is appetitive (see Chapter 1). Referring to the startle and the fMRI studies here, the painful electric shock may have first induced an aversive state, followed by an appetitive state (the relief) and very probably the backward conditioned stimulus (presented after the painful shock) was associated with such an "appetitive" State B. It is to be noted however that when the backward CS+ was presented just after the shock, fear responses (i.e., the startle response) were potentiated just as in the presence of the forward CS+. Well then, how is this finding explicable according to Solomon's theory? A model which matches the potentiation and the attenuation of startle amplitude after backward conditioning is the sometime opponent theory (SOP) suggested by Wagner (1981). The SOP theory assumes that a stimulus may induce two states of activation: the A1 state and the A2 state. Any stimulus is assumed to excite a set of elements, the A1 state implicates that these elements are high activated, whereas the A2 state implicates that the stimulus elements are less activated. Wagner (1981) linked the activation of the elements with attentional processes. Thus, in one case (A1 state) the elements of the stimulus are the focus of attention and in the other case (A2 state) they are at the margin of the attention. If two stimuli are in A1 state, then both of them are associated and an excitatory response is induced. If, however, one stimulus is in A1 state and the other is in A2 state, the association will be inhibitory, because the elements of the stimulus in A1 state are associated with those elements in A2 state. Accordingly, in the startle study, when backward CS+ was presented just after or just before the aversive US, an excitatory response was elicited, i.e. the potentiation of startle response. Suitably, both CS+ and US could have been in the A1 state simultaneously. Consequently such an association is excitatory and fear responses (i.e., the startle response) are potentiated in the presence of delay forward and delay backward conditioning. Similarly, Mallan et al. (2008) found increased startle amplitude in the presence

of pictures conditioned with aversive pictures (aversive US) independently of their temporal sequence (i.e., forward or backward conditioning). The authors suggested that the potentiation of the startle reflex in the presence of the conditioned picture after both forward and backward conditioning is due to the attention allocation to motivationally salient stimuli. Instead, when CS+ was presented a few second after US, startle amplitude was attenuated. This is probably due to the fact that US is not longer the focus of attention and its "memory" is less active. Consequently an inhibitory association is induced, as the attenuation of the startle amplitude suggests.

In his theory, Wagner (1981) gave a good explanation of why a stimulus presented after a biologically significant event may either enhance or lessen fear responses. However, he does not clarify the "affective aspects" of such a modulation. Thus, if it is clear that the potentiation of the startle amplitude is due to an activation of the defensive system and that this kind of activation is probably induced by the simultaneous A1 state of CS+ and US. It is less clear what the attenuation of the startle amplitude implicates. On the one hand, it suggests that the elements of CS+ and US are in A1 and A2 states respectively and therefore the conditioned responses are inhibited. On the other hand, referring to Lang (1995), appetitive and defensive systems operate in an opponent fashion, meaning that by the activation of one system, the other is inhibited. Therefore, the attenuation of the startle should indicate less activation of the defensive system and this lets presume that the appetitive system is now active. In line with this supposition, Dickinson (1979) suggested that an inhibitor of the defensive system operates as the excitor of the appetitive system. Consistently, a broad literature in both animals and humans reports inhibition of startle reflex by pleasant stimuli. Thus, startle amplitude was attenuated in the presence of positive and high arousing pictures (Bradley, et al., 2001; Lang, 1995), safety signals (Jovanovic, Keyes, Fiallos, Myers, Davis, & Ducan, 2005) or cues associated with appetitive reinforcements like food (Schmidt, Koch, & Schnitzler, 1995). Furthermore, findings from neuroscience highlighted that cortical structures implicated in reward processing are also implicated in startle response attenuation. For example Koch and colleagues (1996) found that appetitive conditioning was disrupted if the NAcc was lesioned. Concordant with this evidence, the striatum (the ventral part of which is the NAcc) was strongly activated by backward CS+. Moreover, PCC was strongly activated in response to the backward CS+. Interestingly, PCC has been found to specifically process behavioral responses to a reward (Tabuchi, et al., 2005). Therefore, since the striatum and the PCC are cortical areas implicated in reward processing (O´Doherty, et al., 2004; Schultz

2007; Schultz, et al., 1997; Tabuchi, et al., 2005), this finding suggests that backward CS+ acquired appetitive properties.

Theoretically, the opponent process theory of acquired motivation (Solomon, 1980) and the SOP theory (Wagner, 1981) seem to implicate completely different processes (in one case affective processing and, attentional processing in the second), but might not exclude themselves reciprocally. In other words, presenting an aversive stimulus such as a painful electric shock elicits its elements to the A1 state. At the same time, the shock should have initiated an *a* process inducing the unpleasant State A. When the shock has elapsed for a few seconds, its elements pass from the A1 state to the A2 state. And at the same time, the *a* process has been lessening its intensity while the *b* process increases its intensity inducing the State B which is pleasant. It might be that the high activated A1 state conceived by Wagner corresponds to the State A conceived by Solomon. Thus, the painful electric shock primes the defensive system (State A) and activates the US elements (A1 state). If the backward conditioned stimulus is presented just after the shock, the defensive system would still be active as well as the elements of US. Consequently, the association between backward CS+ and the US may have been excitatory and aversive as suggested by the potentiation of startle response. However, if the backward CS+ is presented few seconds after the aversive event, it is possible to think that the *b* process would have induced the appetitive State B and that the elements of US had passed in the A2 state. As a consequence the association between backward CS+ and the US may have been inhibitory and appetitive as suggested by the attenuation of the startle amplitude. Perhaps, the A2 state and the state B need some time to reach their maximum. Indeed, attenuation of fear responses as well as activation of rewarding neural circuits were found only when six seconds elapsed between US onset and CS+ onset. Coherently, Tanimoto et al. (2004) found greater approach behaviors when the backward conditioned odor followed the electric shock after some seconds (see Figure 1.1). And Cunningham et al. (2002) hypothesized that the injection of ethanol had short duration aversive effect that was followed by a longer lasting rewarding effect. When the animals received the intragastric injection of ethanol, then they were put in a chamber (the CS+) for 5 min. Presumably, the initial aversive effect dissipated and was replaced by the rewarding effect which was then associated with the chamber. As a consequence of this association animals showed CCP.

These results may also be consistent with other conditioning theories (Dickinson, 1979; Konorski, 1957). Konorski (1957) observed that a prior appetitive conditioned stimulus has inhibitory or antagonist effects on the subsequent development of a defensive response.

Elaborating Konorski's observations, Dickinson (1979) claimed that a cue associated with an aversive US may simultaneously elicit the defensive system and inhibit the appetitive system. Thus, a stimulus that is the excitor of a motivational system is also the inhibitor of the opponent system. Such a cue leads to the *emission of preparatory behavior* (p. 211) such as withdrawal when associated with a threat. The omission of a threat and the omission of a reward are as important as their presentation for the organism's survival. The authors highlighted that a stimulus signaling the absence of an aversive event has similar inhibitory effects on the defensive system as a cue signaling a reward. Hence, the backward CS+ might signal the termination (or the absence) of the painful electric shock and consequently have excitatory effects on the appetitive system.

At this point, some reflections on the subjective ratings are necessary to understand the physiological results better and to interpret the results completely. Differently from animals, humans are complex organisms with explicit cognition about associations. Therefore, consciously expecting a danger may involve different processes and therefore modulate the conditioned responses differently than when participants had not such knowledge (Mechias, Etkin, & Kalisch, 2010). In other words, if participants are aware about the association between CS+ and US and knowingly expect the aversive response, they may also react differently from those who do not consciously expect the danger. Participants in the startle and in the fMRI study could correctly verbalize the association between CS+ and US after forward and backward conditioning. Hence, this conscious and explicit awareness could have modulated their ratings for valence and arousal. Indeed, the participants rated the stimulus associated with the aversive event as "emotionally" negative and high arousing. This finding suggests that no matter when the individuals received the electric shock, such an association induces unpleasant feelings. In this case, however, Konorski (1957) and Dickison (1979) proposal may not longer clarify why participants reacted to the backward CS+ with appetitive responses and reported it as aversive. Thus, these authors asserted that the conditioned stimulus leads to a *preparatory behaviors*, i.e. CS+ is signaling the approach or the omission of a biologically significant event and therefore primes appropriate responses according to the information. The backward CS+ in the startle and fMRI studies seems to signal the termination of pain, but not its absence. Indeed, participants could report the presence of US by the presence of CS+. So how is it explainable that the participants correctly reported the association between the shock and the CS+, if they expect the absence of the shock by backward CS+? Solomon (1980) and Wagner (1981) theories can explain this opposite aspect to the conditioned responses. Thus, these models assert that it is thank to the aversive event

that such an appetitive reaction is induced. Therefore, they do not exclude an explicit association with the shock and an implicit appetitive response. However, the two authors do not make any distinction between implicit and explicit responses. But their models might just account for the implicit associative processes. Interestingly, dual-process theories (Bechara, 2005; Strack, & Deutsch, 2004) proposed that human behavior is determined by the output of two systems, an impulsive, implicit system working by associative principles and a reflective, explicit system following decisions on the basis of knowledge about facts and values. These systems operate in a synergic or in an antagonistic fashion. Hence, here the participants might have responded on the basis of the reflective system in regard to the valence and the arousal ratings, whereas they might have responded on the basis of the impulsive system in regard to the startle response and the activation of the striatum and the PCC. Therefore, the backward CS+ might have then been paired with the appetitive system according to (impulsive) associative principles, but the contingency awareness (i.e., the explicit knowledge about the association between CS+ and US) may have induced aversive ratings.

Few further considerations are needed in regard to the attentional processes behind event learning. I have already mentioned that to be at the focus of attention or at the margin may induce excitatory or inhibitory responses. Furthermore, several studies showed that the more salient a stimulus is the more attention-getting it is (Bradley, et al., 2003; Bradley, et al., 2006; Keil, et al., 2002; Moratti, et al., 2006; Öhman, & Mineka, 2001). This effect has been related to motivated attention in which relevant stimuli naturally and perhaps automatically arouse and direct attentional resources (Lang, et al., 1990) Accordingly, both forward and backward conditioned stimuli become more attention-getting through the association with biologically significant unconditioned events and therefore they received more attention afterwards. Most importantly, attentional processes did not seem to be caught by the stimulus per se, but only by those seconds that were most informative about the electric shock. Indeed, the ssVEP amplitude (as the index of attentional processes) was higher during the last seconds of forward CS+, but during the first seconds of backward CS+. Presumably on the basis of these observations, both the onset and the termination of an aversive event are biologically significant for organism survival, therefore requiring more attentional resources.

### 5.1. Outlook

Thus far, much of the research done in humans using classical conditioning has replicated existing animal models. And animal models have provided useful descriptions and hypotheses for investigation in humans. However, humans are complex organisms capable of cognitions

and this has permitted the extension of the animal results to social-cultural means of learning and cognitive strategies (Delgado, et al., 2006). Likewise, the results provided here further support animal models but also added striking inputs. In fact, humans showed appetitive responses after backward association as animals did, but the conscious and reflective responses dissociated from the automatic reactions. Such a rift between explicit and implicit valence of the backward CS+ might contribute to the understanding of psychopathologies like anxiety disorders (Bouton, et al., 2001; Himadi, 1987; Michael, Blechert, Vrieds, & Wilhelm, 2007) or drug addiction (Koob, & Moal, 1997; Koob, & LeMoal, 2001; Weiss, 2005). Evidently, an interpretation of the results in terms of relevance for psychiatric disorders would be purely speculative; though this does not preclude the possibility of additional reflection.

Notably, the results of this thesis seem to fit the proposals of Koob and LeMoal (2001; 1997) and Weiss (2005) regarding the pathogenesis of drug addiction very well. The authors suggested that the transition from drug use to drug addiction depends on many factors such as availability, genetics, history of drug use, stress and life events. Most definitions of drug addiction include overwhelming involvement with the use of a drug (compulsive use), loss of control over drug intake and narrowing of the number of different behavioral responses toward drug seeking (World Health Organization, 1992). According to these authors, drug addiction represents an allostatic state in the brain reward system. Thus, an organism tends to maintain equilibrium in all of its systems, including the brain. Some environmental factors may challenge this homeostasis and consequently the organism will try to restore the previous equilibrium. Continuous intake of the drug may impair the natural reward system and a process defined allostasis takes place. Allostasis is defined as a state of chronic deviation of the regulatory system from its normal state of operation with establishment of a new set point (Koob, et al., 2001). In other word, the organism needs external “help” (i.e., the drug) to reach the desiderated homeostatic state. And it is based on these observations that Mr. Koob, Mr. Weiss and their colleagues suggested that the abstinence from the drug can have motivational significance in maintaining drug seeking behaviors. Abstinence includes various negative emotions such as dysphoria, depression, irritability and anxiety and in order to relieve these negative emotional states, the person will seek the drug. Furthermore, Bechara (2005) suggested that addiction is due to the imbalance between the impulsive and the reflective system. Thus, drug abusers showed impairments in top-down control mechanisms (i.e., the reflective system) and a facilitated bottom-up control (i.e., the impulsive system). Hence, the drug intake might be reinforced “twice”, i.e. by the positive effects of the drug and by the positive after-effect induced by the drug intake. Curiously, backward association with an

aversive event indicated a dissociation between the impulsive and reflective system. Further drug theories proposed that the environmental cues (CS+) would be associated with the drug intake (appetitive US) and consequently the presence of such a cue would produce those automatic changes in the organism as expectation of the drug being received (for a review see O'Brien, Childress, Ehrman, & Robbins, 1998). But what would it implicate, if the unconditioned stimulus is not the drug, but the negative feeling preceding its intake? Would therefore the termination of the negative feeling due to the drug intake have those reinforcing properties? Nicely, the results of this thesis seem to support that termination of an aversive event has reinforcing and appetitive motivational properties. But this need not be the answer to this question. Further studies are needed to deepen the role of backward association in drug addiction.

Anxiety disorders have been defined as an apprehensive anticipation of future danger, often accompanied by somatic symptoms of tension or feeling of dysphoria and the focus of anticipated danger can be internal or external (Bouton, et al., 2001). Fear conditioning may be considered in the pathogenesis of anxiety disorders (Bouton, et al., 2001; Mineka, & Oehlberg, 2008). In other words, once fear conditioning has occurred, CS+ singles the imminence of the danger and elicits a constellation of responses which may depend on how close in time and space the danger is. Similarly, an organism interrupts its daily duty to false alarms (i.e., such people fear a danger when there is nothing to fear) and "learn" to be constantly alarmed. In particular, people suffering of panic disorder (PD) experienced an abrupt experience of intense fear or discomfort accompanied by a number of physical and mental symptoms several times. In addition, the individuals develop substantial anxiety or concern over the possibility of having another attack or about the implications of the attack or its consequences, e.g. the attack leads to heart attack, to "going crazy" or to "losing control". It has been shown that PD patients did differ from healthy control participants in regard to their fear acquisition and importantly to fear extinction that is they showed stronger fear responses to CS+ and less extinction of fear responses (Himadi, 1987; Lissek, et al., 2005; Michael, et al., 2007). Interestingly, Bouton, et al. (2001) argued that panic attacks are strong conditioning episodes during which feelings of anxiety and panic become associated with exteroceptive (e.g., open places) and interoceptive (e.g., dizziness) cues present during the attacks. Subsequent encountering of these cues can trigger feelings of anticipatory anxiety, which may serve to exacerbate the next panic attack or the panic itself. However, it is necessary here to consider not only the signal of an aversive event, but also its termination. Previous studies highlighted that both the coming and the termination of an aversive event are

informative and induce conditioning (Himadi, 1987). Furthermore, a cue signaling threat termination induces the opposite behaviors than a cue signaling that the threat is coming that is the former induces approach (Seymour, et al., 2005; Solomon, 1980) and the latter avoidance. Hence, according to Bouton's proposal, the panic attack may be considered the aversive US, but what would this implicate if we consider backward associations? Could the termination of a panic attack be an appetitive reinforcer? Undoubtedly, these questions need to be answered. But panic disorder patients are frequently characterized by being attracted to "safety" stimuli such as physician or ambulance. Therefore, according to the results of this thesis, the PD patients may for example have associated the physician with the appetitive after-effect of a panic and be willing to stay in constant contact with him/her, slowly loosing independence as individual. Normally, the therapy for anxiety disorder in general and for PD in particular implicates new learning, i.e. the patients try to learn that CS+ is not as aversive as they learned by means of associative processes (Bouton, 2002; Schiller, Monfils, Raio, Johnson, LeDoux, & Phelps, 2009). Interestingly, PD patients have particularly reduced ability in this "new learning" (Michael, et al., 2007). Hence, if it is true that this kind of mechanism follows associative principles disorder, then it would be plausible to consider a therapeutic approach that involves "new" extinction processes regarding the backward association between the physician (the backward CS+) and the appetitive after-effect (the appetitive US) of a panic.

Another challenging hypothesis asserts that to make a decision, to select an action from a set of available options and to obtain an optimal outcome requires high order cognitive functions that must be able to appraise the needs of the individual as well as the environmental conditions (Paulus, 2007). Hence, before making a decision, the individual should consider both its interoceptive and its exteroceptive aspects to re-establish the original homeostatic state (for a further explanation see Craig, 2003). An altered homeostatic state might then determine an altered assessment and formation of preferences, an altered selection and execution of the actions and an attenuated or exaggerated experience or evaluation of an outcome. It has been suggested that the insula may have a role in integrating visceral and affective signals with cognitive processes (Büchel, et al., 1998; Paulus, & Stein, 2006). Hence, an altered insula functioning might induce decision making after the wrong consideration of the possibilities. In terms of Bechara's hypothesis, it would be that the reflective and cognitive top-down control does not work appropriately anymore, and the behaviors are based on impulsive principles. Therefore, malfunctioning of the insula might also have a role in the development of anxiety disorders. Thus, some individuals have the

tendency to view interoceptive sensations as dangerous or threatening especially people with anxiety disorders (Paulus, & Stein, 2006). Hence, maladaptive functioning of the insula may permit hyperactivation of automatic responses such as the increase of anxious affects, worrisome thoughts and avoidance behaviors, without permitting the cognitive system to top-down regulate such hyperactivity. Interestingly, the high arousal ratings for the backward CS+ correlated with the high activation in the insula. Thus, the subjective appraisal of the backward CS+ dissociated from the physiological responses and here again the insula seems to be the inter-connective neural structure between the conscious and unconscious appraisal. Therefore, it would be really interesting to test whether such dissociation as emerged in these studies would also have a role in the development of anxiety disorders and which kind of consequence maladaptive functioning of the insula might have in backward associations. Would the malfunctioning of the insula potentiate the appetitive after-effect of the backward CS+?

In summary, many questions still remain open and further studies should be done. However, an important result has been found here: Backward conditioning occurs in humans and backward association is fundamentally different from forward association. Although forward and backward conditioned stimuli acquired new properties thank to their association with a biologically significant event and became more salient (attention-getting) by means of this association, the acquired meaning is completely different. Forward CS+ signals the coming of an aversive event and involves the defensive motivational system; whereas the backward CS+ signals the termination of an aversive event and involves the appetitive motivational system. In line with the results of these studies (the startle study, the fMRI study and the ssVEP study), forward CS+ becomes a predictor for punishment and backward CS+ for relief. On this basis, it is reasonable to speak of punishment and relief learning respectively. Besides this, it would be really interesting to study how event learning would modulate behavioral responses like approach (i.e., to choose or to pick up an object which is backward associated with an electric shock) or avoidance (i.e., to do not choose or to throw away an object which is forward associated with an electric shock). In fact, the affective state primes a behavioral response (implicit system), but the individual is aware of what he/she is doing (explicit system). Moreover, backward associations might give crucial hints into the mechanisms underlying psychiatric disorders (as I delineated in the outlook) and the dissociation between the implicit and explicit systems might improve the understanding of such disorders.

**Glossary**

| *Abbreviations* | *Definition* |
|---|---|
| **ACC** | Anterior Cingulate Cortex |
| **BLA** | Basolateral nucleus of Amygdala |
| **BOLD** | Blood-Oxygen-Level-Dependent |
| **CC** | Cingulate Cortex |
| **CeA** | Central nucleus of Amygdala |
| **CR** | Conditioned Response, that is the responses induced by a conditioned stimulus after classical conditioning |
| **CS-** | Conditioned Stimulus that is the stimulus presented during the conditioning, but never associated with the unconditioned stimulus |
| **CS+** | Conditioned Stimulus, that is the stimulus presented during conditioning and always associated with the unconditioned stimulus |
| **DA** | Dopamine |
| **EEG** | Electroencephalogram |
| **FFT** | Fast Fourier Transformation |
| **fMRI** | functional Magnetic Resonance Imaging |
| **GAD** | Generalized Anxiety Disorder |
| **HR** | Heart Rate |
| **IAPS** | International Affective Picture System (Lang, 1999) |
| **ISI** | Inter-Stimulus Interval, that is the time window between the unconditioned stimulus onset and the conditioned stimulus onset |
| **ITI** | Inter-Trial Interval, that is the time window between one stimulus's offset and the next stimulus's onset |
| **LD** | Latero-Dorsal nuclei |
| **LTD** | Long-Term Depression |
| **LTP** | Long-Term Potentiation |
| **MID** | Monetary Incentive Delay task. This task consists in the presentation of three colored shapes, one shape signaled rewarded response (i.e., when participants responded, it won some monetary amount), another shape signaled unrewarded response (i.e., even if participant responded, it did not win anything) and the third shape signaled no-response requirement (i.e., |

| | |
|---|---|
| | participant did not have to response) |
| **NAcc** | Nucleus Accumbens |
| **NEW** | The novel control stimulus that is the additional visual stimulus presented only during test phase (i.e., the extinction) |
| **NMDA** | N-Methyl-D-Asparate |
| **OFC** | Orbito-Frontal Cortex |
| **PCC** | Posterior Cingulate Cortex |
| **PD** | Panic Disorder |
| **PE** | Prediction Error |
| **PFC** | Pre-Frontal Cortex |
| **PL** | Punishment Learning, that is the forward conditioning paradigm in which the unconditioned stimulus in an aversive event |
| **PMC** | Pre-Motor Cortex |
| **PnC** | Caudal Pontine reticular nucleus |
| **PTSD** | Post-Traumatic Stress Disorder |
| **RL** | Relief Learning, that is the backward conditioning paradigm in which the unconditioned stimulus is an aversive event |
| **ROI** | Region Of Interest |
| **SCR** | Skin Conductance Response |
| **SMA** | Supplementary Motor Area |
| **SOP** | Sometime Opponent Process theory (Wagner, 1981) |
| **US** | Unconditioned Stimulus, that is a stimulus biologically salient, which induces unconditioned responses without needing learning (e.g., an electric shock) |
| **vmPFC** | ventromedial Pre-Frontal Cortex |
| **ssVEF** | steady-state Visual Evoked magnetic Field |
| **ssVEP** | steady-state Visual Evoked Potential |
| **STDP** | Spike-Timing-Dependent Plasticity |
| **VTA** | Ventral Tegmetal Area |
| **5-TH** | 5-Hydroxytryptamine or serotonin |

| *Terminology* | *Definition* |
|---|---|
| **APPETITIVE conditioning** | Classical conditioning procedure in which CS+ is associated with an appetitive (rewarding) event, e.g. sweet food or money |
| **BACKWARD conditioning** | Classical conditioning procedure in which CS+ follows US (US → CS+) |
| **CONTIGENCY AWARENESS** | The subject can verbalize the reinforcement contingencies in the experiment, that is the knowledge about the association between CS+ and US |
| **DELAY conditioning** | Classical conditioning procedure in which the US offset and CS+ offset overlap, that is CS+ and US coterminate. Hippocampus-independent task |
| **FEAR or AVERSIVE conditioning** | Classical conditioning procedure in which the CS+ is associated with an aversive (generally painful) event, e.g. electric shock |
| **FORWARD conditioning** | Classical conditioning paradigm in which CS+ precedes US (CS+ → US) |
| **TRACE conditioning** | Classical conditioning procedure which involves a temporal gap between CS+ offset and US onset. The participant's contingency awareness is a prerequisite for CR to occur. Hippocampus-dependent task |

# 6. Reference

Abbott, L.F., & Nelson, S.B. (2000). Synaptic plasticity: taming the beast. *Nature Neuroscience, 3*, 1178-1183.

Adolph, R. (2002). Neural system for recognizing emotion. *Current Opinion in Neurobiology, 12*, 169-177.

Adolph, R., Tranel, D., Damasio, H., & Damasio, A. (1994). Impaired recognition of emotion in facial expressions following bilateral damage to the human amygdala. *Nature, 372*, 669-672.

Aragona, B.J., Liu, Y., Yu, Y.J., Curtis, T., Detwiler, J.M., Insel, T.R., & Wang, Z. (2006). Nucleus accumbens dopamine differentially mediates the formation and maintenance of monogamous pair bonds. *Nature Neuroscience, 9*, 133-139.

Arcediano, F., Escobar, M., & Miller, R.R. (2003). Temporal integration and temporal backward associations in human and nonhuman subjects. *Learning & Behavior, 31*, 242-256.

Ashburner, J., & Friston, K.J. (2005). Unifield segmentation. *NeuroImage, 26*, 839-851.

Barlow, D.H. (2000). Unraveling the mysteries of anxiety and its disorders from the perspective of emotion theory. *American Psychologist 55*, 1247-1263.

Bassareo, V., Luca, D., M.A., & Di Chiara,G. (2002). Differential expression of motivational stimulus properties by dopamine in nucleus accumbens shell versus core and prefrontal cortex. *The Journal of Neuroscience 22*, 4709-4719.

Bechara, A. (2005). Decision making, impulse control and loss of willpower to resist drugs: a neurocognitive perspective. *Nature Neuroscience, 8*, 1458-1463.

Bechara, A., Damasio, H., & Damasio, A.R. (2000). Emotion, decision making and the orbitofrontal cortex. *Cerebral Cortex, 10*, 295-307.

Bechara, A., Tranel, D., Damasio, H., Adolphs, R., Rockland, C., & Damasio, A.R. (1995). Double dissociation of conditioning and declarative knowledge relative to the amygdala and hippocampus in humans. *Science, 269*, 1115-1118.

Berthier, M., Starkstein, S., & Leiguarda, R. (1988). Asymbolia for pain: A sensory-limbic disconnession syndrome. *Annals of Neurology, 24*, 41-49.

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.

Bouton, M.E., Barlow, D.H., & Mineka, S. (2001). A modern learning theory perspective on the etiology of panic disorder. *Psychological Review, 108*, 4-32.

Bradley, M.M., Codispoti, M., Cuthbert, B.N., & Lang, P.J. (2001). Emotion and motivation I: defensive and appetitive reactions in picture processing. *Emotion, 1*, 276-298.

Bradley, M.M., Codispoti, M., & Lang, P.J. (2006). A multi-process account of startle modulation during affective perception. *Psychophysiology, 43*, 486-497.

Bradley, M.M., Moulder, B., & Lang, P.J. (2005). When good things go bad. *Psychological Science, 16*, 468-473.

Bradley, M.M., Sabatinelli, D., Lang, J.P., Fitzsimmons, J.R., King, W., & Desai, P. (2003). Activation of the visual cortex in motivated attention. *Behavioral Neuroscience, 117*, 369-380.

Brischoux, F., Chakraborty, S., Brierley, D.I., & Ungless, M.A. (2009). Phasic excitation of dopamine neurons in ventral VTA by noxious stimuli. *PNAS, 106*, 4894-4899.

Bromm, B. (2004). The involvement of the posterior cingulate gyrus in phasic pain processing of humans. *Neuroscience Letters, 361*, 245-249.

Buchanan, S.L., & Powell, D.A. (1982). Cingulate cortex: its role in Pavlovian conditioning. *Journal of Comparative and Physiological Psychology, 96*, 755-774.

Büchel, C., & Dolan, R.J. (2000). Classical fear conditioning in functional neuroimaging. *Current Opinion in Neurobiology, 10*, 219-223.

Büchel, C., Morris, J., Dolan, R.J., & Friston, K.J. (1998). Brain systems mediating aversive conditioning: an event-related fRMI study. *Neuron, 20*, 947-957.

Burman, M.A., & Gerwitz, J.C. (2004). Timing of fear expression in trace and delay conditioning measured by fear-potentiated startle in rats. *Learning & Memory, 11*, 205-212.

Cahill, L., & McGaugh, J.L. (1990). Amygdaloid complex lesions differentially affect retention of tasks using appetitive and aversive reinforcement. *Behavioral Neuroscience, 104*, 532-543.

Canli, T., & Lesch, K.P. (2007). Long story short: the serotonin transporter in emotion regualtion and social cognition. *Nature Neuroscience, 10*, 1103-1109.

Cardinal, R.N., Parkinson, J.A., Hall, J., & Everitt, B.J. (2002). Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. *Neuroscience and Behavioral Reviews, 26*, 321-352.

Chang, R.C., Blaisdell, A.P., & Miller, R.R. (2003). Backward conditioning: mediation by the context. *Journal of Experimental Psychology, Animal Behavior Processes, 29*, 171-183.

Chang, R.C., Stout, S., & Miller, R.R. (2004). Comparing excitatory backward and forward conditioning. *The Quartely Journal of Experimental Psychology 57B*, 1-23.

Cheng, D.T., Knight, D.C., Smith, C.N., Stein, E.A., & Helmstetter, F.J. (2003). Functional MRI of human amygdala activity during pavlovian fear conditioning: stimulus processing versus response expression. *Behavioral Neuroscience, 117*, 3-10.

Clark, R.E., & Squire, L.R. (1998). Classical conditioning and brain systems: the role of awareness. *Science, 180*, 77-81.

Coghill, R.C., Sang, C.N., Maisog, J.MA., & Iadarola, M.J. (1999). Pain intensity processing within the human brain: a bilateral, distributed mechanism. *The American Physiological Society*

Cohen, M.X., Axmacher, N., Lenartz, D., Elger, C.E., Sturm, V., & Schlaemfer, T.E. (2009). Neuroelectric signatures of reward learning and decision-making in the nucleus accumbens. *Neuropsychopharmacology, 34*, 1649-1658.

Cole, R.P., Barnet, R.C., & Miller, R.R. (1995). Temporal encoding in trace conditioning. *Animal Learning & Behavior 23*, 144-153.

Cole, R.P., & Miller, R.R. (1999). Conditioned excitation and conditioned inhibition acquired through backward conditioning. *Learning and Motivation, 30*, 129-156.

Cooper, & L.D. (1991). Temporal factors in classical conditioning. *Learning and Motivation, 22*, 129-152.

Cox, S.M., Andrade, A., & Johnsrude, I.S. (2005). Learning to like: a role for human orbitofrontal cortex in conditioned reward. *The Journal of Neuroscience, 25*, 2733-2740.

Craig, A.D. (2003). A new view of pain as a homeostatic emotion. *Trends in Neurosciences, 26*, 303-307.

Cunningham, C.L., Clemans, J.M., & Fidleer, T.L. (2002). Injection timing determines whether intragastric ethanol produces conditioned place preference or aversion in mice. *Pharmacology, Biochemistry and Behavior, 72*, 659-668.

Damasio, A. R. (1994). *Descarte´s error: Emotion, rationally and the human brain.* New York: Grosset Books.

Davis, H., Falls, W.A., Campeau, S., & Kim, M. (1993). Fear-potentiated startle: a neural and pharmacological analysis. *Behavioral Brain Research, 58*, 175-198.

Davis, M. (1998). Anatomic and physiologic substrates of emotion in an animal model. *Journal of Clinical Neurophysiology, 15*, 378-387.

Davis, M. (2006). Neural systems involved in fear and anxiety measured with fear-potentiated startle. *American Psychologist, 61*, 741-756.

Davis, M., & Shi, C. (2000). The amygdala. *Current Biology, 10*, R131.

Davis, M., & Whalen, P.J. (2001). The amygdala: vigilance and emotion. *Molecular Psychiatry, 6*, 13-34.

Daw, N.D., Kakade, S., & Dayan, P. (2002). Opponent interactions between serotonin and dopamine. *Neural Networks, 15*, 603-616.

Dayan, P., & Balleine, B.W. (2002). Reward, motivation, and reinforcement learning. *Neuron, 36*, 285-298.

Delgado, M.R. (2007). Reward-related responses in the human striatum. *Annual of the New York Academy of Sciences, 1104*, 70-88.

Delgado, M.R., Li, J., Schiller, D., & Phelphs, E.A. (2008). The role of the striatum in aversive learning and aversive prediction errors. *Philosophical Transactions of the Royal Society*, 1-14.

Delgado, M.R., Locke, H.M., Stenger, V.A., & Fiez, J.A. (2003). Dorsal striatum responses to reward and punishment: effects of valence and magnitude manipulations. *Cognitive, Affective, & Behavioral Neuroscience, 3*, 27-38.

Delgado, M.R., Olsson, A., & Phelps, E.A. (2006). Extending animal models of fear conditioning to humans. *Biological Psychology, 73*, 39-48.

Devriese, S., Winters, W., Van Dienst, I., & Van den Bergh, O. (2004). Contingency awareness in a symptom learning paradigm: necessary but not sufficient? *Consciouness and Cognition 13*, 439-452.

Dickinson, A., & Dearing, M.F (1979). Appetitive-aversive interactions and inhibitory processes. In Dickinson, A., & Boakes R.A. (Eds.), *Mechanisms of learning and motivation: A memorial volume to Jerzy Konorsky*. New Jersey: Lawrence Erlbaum Associates, Publishers.

Dorris, M.C., & Glimcher, P.W. (2004). Activity in posterior parietal cortex is correlated with the relative subjective desiderability of action. *Neuron, 44*, 365-378.

Drew, P.J., & Abbott, L.F. (2006). Extending the effects of spike-timing-dependent plasticity to behavioral timescales. *PNAS, 103*, 8876-8881.

Dunsmoor, J.E., Bandettini, P.A., & Knight, D.C. (2007). Impact of continuous versus intermittent CS-UCS pairing on human brain activation during pavlovian fear conditioning. *Behavioral Neuroscience, 121*, 635-642.

Ebbinghaus, H. (1885). *Memory, a contribution to experimental psychology*. New York: Teachers College, Columbia University.

Fendt, M., & Fanselow, M.S. (1999). The neuroanatomical and neurochemical basis of conditioned fear. *Neuroscience and Biobehavioral Reviews, 23*, 743-760.

Di Filippo, M., Picconi, B., Tantucci, M., Ghiglieri, V., Bagetta, V., Sgobio, C., Tozzi, A., Parnetti, L., & Clabresi, P. (2009). Short-term and long-term plasticity at corticastriatal sysnapses: Implications for learning and memory. *Behavioral Brain Research, 199*, 108-118.

Fletcher, P.J., & Korth, K.M. (1999). Activation of 5-$HT_{1B}$ receptors in the nucleus accumbens reduces amphetamine-induced enhancement of responding for conditioned reward. *Psychopharmacology, 142*, 165-174.

Fredrikson, M., Wik, G., Fischer, H., & Andersson, J. (1995). Affective and attentive neural networks in humans: a PET study of Pavlovian conditioning. *NeuroReport, 7*, 97-101.

Gazzaniga, M.S., Ivry, R.B., & Mangun, G.R. (2002). *Cognitive neuroscience. The biology of the mind.* New York, London: Norton & Company.

Gläscher, J., Hampton, A.N., & O´Doherty, J.P. (2009). Determing a role for ventromedial prefrontal cortex in encoding action-based value signals during reward-related decision making. *Cerebral Cortex, 19*, 483-495.

Gottfried, J.A., O´Doherty, J., & Dolan, R.J. (2002). Appetitive and aversive olfactory learning in humans studied using event-related functional magnetic resonance imaging. . *The Journal of Neuroscience 22*, 10829-10837.

Gray, M., Kemp, A.H., Silbertein, R.B., & Nathan, P.J. (2003). Cortical neurophysiology of anticipatory anxiety: an investigation utilizing steady state probe topography (SSPT). *NeuroImage, 20*, 975-986.

Grillon, C. (2002). Startle reactivity, and anxiety disorders: aversive conditioning, context and neurobiology. *Biological Psychiatry, 56*, 958-975.

Grillon, C., & Ameli, R. (1998b). Effects of threat of shock, shock electrode placement and darkness on startle. *International Journal of Psychophysiology, 28*, 223-231.

Grillon, C., & Baas, J. (2003). A review of the modulation of the startle reflex by affective states and its application in psychiatry. *Clinical Neurophysiology 114*, 1557-1579.

Grillon, C., Baas, J.M., Cornwell, B., & Johnson, L. (2006). Context conditioning and behavioral avoidance in a virtual reality environment: effect of predictability. *Biological Psychiatry, 60*, 752-759.

Grillon, C., Morgan, C.A., Davis, M., & Southwick, S.M. (1998a). Effects of darkness on acoustic startle in Vietnam veterans with PTSD. *American Journal of Psychiatry, 155*, 812-817.

Hamm, A.O., & Vaitl, D. (1996). Affective learning: awareness and aversion. *Psychophysiology, 33*, 698-710.

Hamm, A.O., & Weike, A.I. (2005). The neuropsychology of fear learning and fear regulation. *International Journal of Psychophysiology, 57*, 5-14.

Hariri, A.R., Mattay, V.S., Tessitore, A., Kolachana, B., Fera, F., Goldman, D., Egan, M.F., & Weinberger, D.R. (2002). Serotonin transporter genetic variation and the response of the human amygdala. *Science, 297*, 400-403.

Hebb, D. (1949). *The organisation of behavior: A neuropsychological theory*. New York: John Wiley and Sons.

Hellstern, F., Malaka, R., & Hammer, M. (1998). Backward inhibitory learning in honeybees: a behavioural analysis of reinforcement processing. *Learning & Memory 4*, 429-444.

Hermann, C., Ziegler, S., Birbaumer, N., & Flor, H. (2000). Pavlovian aversive and appetitive odor conditioning in humans: subjective, peripheral, and electrocortical changes. . *Experimental Brain Research 132*, 203-215.

Heth, C.D. (1976). Simultaneous and backward fear conditioning as a function of number of CS-UCS pairings. *Journal of Experimental Psychology: Animal Behavior Processes 2*, 117-129.

Himadi, W.G. (1987). Safety signals and agoraphobia. *Journal of Anxietx Disorders, 1*, 345-360.

Jensen, J., Smith, A.J., Willeit, M., Crawley, A.P., Mikulis, D.J., Vitcu, I., & Kapur, S. (2007). Separate brain regions code for salience vs. valence during reward prediction in humans. *Human Brain Mapping, 28*, 294-302.

Johnson, A., Meer, v. d., M, & Redish, D. (2007). Integrating hippocampus and striatum in decision-making. *Current Opinion in Neurobiology, 17*,692-697.

Jovanovic, T., Keyes, M., Fiallos, A., Myers, K.M., Davis, M., & Ducan, E.J. (2005). Fear potentiation and fear inhibition in a human fear-potentiation startle paradigm. *Biological Psychiatry, 57*,1559-1564.

Junghöfer, M., Bradley, M.M., Elbert, T.R., & Lang, P.J. (2001). Fleeting images: a new look at early emotion discrimination. *Psychophysiology, 38*, 175-178.

Keil, A., Bradley, M.M., Hauk, O., Rockstroh, B., Elbert, T., & Lang, P.J. (2002). Large-scale neural correlates of affective picture processing. *Psychophysiology, 39*, 641-649.

Keil, A., Gruber, T., Müller, M.M., Moratti, S., Stolarova, M., Bradley, M.M., & Lang, P.J. (2003). Early modulation of visual perception by emotional arousal: evidence from steady-state visual evoked brain potentials. *Cognitive, Affective, & Behavioral Neuroscience, 3*, 195-206.

Keil, A., Moratti, S., Sabatelli, D., Bradley, M.M., Lang, P.J. (2005). Additive effects of emotional content and spatial selective attention on electrocortical facilitation. *Cerebral Cortex, 15*, 1187-1197.

Keil, A., Sabatinelli, D., Ding, M., Lang, P.J., Ihssen, & Heim, S. (2009). Re-entrant projections modulate visual cortex in affective perception: evidence from granger causality analysis. *Human Brain Mapping, 30*, 532-540.

Kenser, R.P., & Hopkins, R.O. (2006). Mnemonic functions of the hippocampus: a comparison between animals and humans. *Biological Psychology, 73*, 3-18.

Kiebel, S., & Holmes, A.P. (2003). The general linear model. In Zeki, S., Ashburner, J.T., Penny, D., Frackowiak, R.S.J., Friston, K.J., Frith, C.D., Dolan, R.J., Price & C.J. (Eds.), *Human brain function* (pp. 725-760). Oxford: Academic Press.

Kim, J.J., & Jung, M.W. (2006). Neural circuits and mechanisms involved in Pavlovian fear conditioning: a critical review. *Neuroscience and Biobehavioral Reviews 30*, 188-202.

Kirsch, P., Schienle, A., Stark, R., Sammer, G., Blecker, C., Walter, B., Ott, U., Burkart, J., & Vaitl, D. (2003). Anticipation of reward in a nonaversive differential conditioning paradigm and the brain reward system: an event-related fRMI study. *NeuroImage, 20*, 1086-1095.

Kluchen, T., Kagerer, S., Schweckendiek, J., Tabbert, K., Vaitl, D., & Stark, R. (2009a). Contingency learning in human fear conditioning involves the ventral striatum. *Human Brain Mapping*.

Kluchen, T., Kagerer, S., Schweckendiek, J., Tabbert, K., Vaitl, D., Stark, R. (2009b). Neural, electrodermal and behavioural response patterns in contingency aware and unaware subjects during a picture-picture conditioning paradigm. *Neuroscience, 158*, 721-731.

Kluver, H., & Bucy, P.C. (1937). "Psychic blindness" and other symptoms following bilateral temporal lobectomy in rhesus monkeys. *American Journal of Physiology, 119*, 352-353.

Knight, D.C., Nguyen, H.T., & Bandettini, P.A. (2003). Expression of conditional fear with and without awareness. *PANAS 100*, 15280-15283.

Knight, D.C., Waters, N.S., & Bandettini, P.A. (2009). Neural substrates of explicit and implicit memory. *NeuroImage, 45*, 208-214.

Knight, D. C., Cheng, D.T., Smith, C.N., Stein, E.A., & Helmstetter, F.J. (2004). Neural substrates mediating human delay and trace fear conditioning. *The Journal of Neuroscience 24*, 218-228.

Knutson, B., Adams, C.M., Fong, G.W., & Hommer, D. (2001a). Anticipation of increasing monetary reward selectively recruits nucleus accumbens. *The Journal of Neuroscience, 21*, RC159.

Knutson, B., Fong, G.W., Adams, C.M., Varner, J.L., & Hommer, D. (2001b). Dissociation of reward anticipation and outcome with event-related fRMI. *NeuroReport, 12*, 3683-3687.

Koch, M. (1999). The neurobiology of startle. *Progress in Neurobiology, 59*, 107-128.

Koch, M., Schmid, A., & Schnitzler, H. (1996). Pleasure-attenuation of startle is disrupted by lesions of the nucleus accumbens. *NeuroReport, 7*, 1442-1446.

Konorski, J., & Szwejkowska, G. (1959). Reciprocal transformations of heterogeneous conditioned reflexes. *Acta Biologiae Experimentalis, 16*, 95-113.

Koob, G.F., & LeMoal, M. (2001). Drug addiction, dysregualtion of reward, and allostasis. *Neuropsychopharmacology, 24*, 97-129.

Koob, G.F., & Le Moal, M. (1997). Drug abuse: Hedonic homeostatic dysregualtion. *Science, 278*, 52-58.

LaBar, K.S., Gatenby, J.C., Gore, J.C., LeDoux, J.E., & Phelps, E.A. (1998). Human amygdala activation during conditioned fear acquisition and extinction: a mixed-trial fRMI study. *Neuron, 20*, 937-945.

LaBar, K.S., LeDoux, J.E., Spencer, D.D., & Phelps, E.A. (1995). Impaired fear conditioning following unilateral temporal lobectomy in humans. *The Journal of Neuroscience, 15*, 6846-6855.

Lane, R.D., Fink, G.R., Chau, P.M.-L., & Dolan, R.J. (1997). Neural activation during selective attention to subjective emotional responses. *NeuroReport, 8*, 3969-3972.

Lang, P.J. (1995). The emotion probe. Studies of motivation and attention. . *American Psychologist 50*, 372-385.

Lang, P.J., Bradley, M.M., & Cuthbert, B.N. (1990). Emotion, attention, and startle reflex. . *Psychological Review 97*, 377-395.

Lang, P.J., Bradley, M.M., & Cuthbert, B.N. (1998). Emotion motivation and anxiety: brain mechanisms and psychophysiology. *Biological Psychiatry, 44*, 1248-1263.

Lang, P.J., Davis, M., Öhman, & A. (2000). Fear and anxiety: animal models and human cognitive psychophysiology. *Journal of Affective Disorders, 61*, 137-159.

LeDoux, J.E. (1995). Emotion: clues from the brain. *Annual Review of Psychology 46*, 209-235.

Leknes, S., & Tracey, I. (2008). A common neurobiology of pain and pleasure. *Nature Reviews. Neuroscience, 9*, 314-320.

Lipp, O.V., Sheridan, J., & Siddle, D.A.T. (1994). Human blink startle during aversive and nonaversive Pavlovian conditioning. *Journal of Experimental Psychology, Animal Behavior Processes 20*, 380-389.

Lissek, S., Pine, D.S., & Grillon, C. (2006). The *strong situation*: A potential impediment to studying the psychobiology and pharmacology of anxiety disorders. *Biological Psychology, 72*, 265-270.

Lissek, S., Powers, A.S., McClure, E.B., Phelps, E.A., Woldehawariat, G., Grillon, C., & Pine, D.S. (2005). Classical fear conditioning in the anxiety disorders: a meta-analysis. *Behavior Research and Therapy 43*, 1391-1424.

Maddock, R.J. (1999). The retrosplenial cortex and emotion: new insights from functional neuroimaging of the human brain. *Trends in Neurosciences, 22*, 310-316.

Maldjian, J.A., Laurienti, P.J., & Burdette, J.H. (2004). Precentral gyrus discrepancy in electronic versions of the Talairach atlas. *NeuroImage, 21*, 450-455.

Maldjian, J.A., Laurienti, P.J., Kraft, R.A., & Burdette, J.H. (2003). An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *NeuroImage, 19*, 1233-1239.

Mallan, K.M., Lipp, O.V., & Libera, M. (2008). Affect, attention, or anticipatory arousal? Human blink startle modulation in forward and backward affective conditioning. *International Journal of Psychophysiology, 69*, 9-17.

Manns, J.R., Clark, R.E., & Squire, L.R. (2000). Parallel acquisition of awareness and trace eyeblink classical conditioning. *Learning and Memory, 7*, 267-272.

Maren, S. (2001). Neurobiology of pavlovian fear conditioning. *Annual Review Neuroscience, 24*, 897-931.

Martin-Soelch, C., Linthicum, J., & Ernst, M. (2007). Appetitive conditioning: neural bases and implications for psychopathology. *Neuroscience and Biobehavioural Reviews, 31*, 426.440.

McCoy, A.N., Crowley, J.C., Haghighian, G., Dean, H.L., & Platt, M.L. (2003). Saccade reward signals in posterior cingulate cortex. *Neuron, 40*, 1031-1040.

McCoy, A.N., & Platt, M.L. (2005). Risk-sensitive neurons in macaque posterior cingulate cortex. *Nature Neuroscience, 8*, 1220-1227.

Mechias, M.L., Etkin, A., & Kalisch, R. (2010). A meta-analysis of instructed fear studies: Implications for conscious appraisal of threat. *NeuroImage, 49*, 1760-1768.

Menon, M., Jensen, J., Vitcu, I., Graff-Guerrero, A., Crawley, A., Smith, M.A., & Kapur, S. (2007). Temporal difference modelling of the blood-oxygen level dependent response during aversive conditioning in humans: effects of dopaminergic modualtion. *Biological Psychiatry, 62*, 765-772.

Michael, T., Blechert, J., Vrieds, J.M., & Wilhelm, F.H. (2007). Fear conditioning in panic disorder: enhanced resistance to extinction. *Journal of Abnormal Psychology, 116*, 612-617.

Milad, M.R., Quirk, G.J., Pitman, R.K., Orr, S.P., Fischl, B., & Rauch, S.L. (2007). A role for the human dorsal anterior cigulate cortex in fear expression. *Biological Psychiatry, 62*, 1191-1194.

Mineka, S., & Oehlberg, K. (2008). The relevance of recent developments in classical conditioning to understanding the etiology and maintenance of anxiety disorders. *Acta Psychologica, 127*, 567-580.

Mineka, S., & Zinbarg, R. (2006). A contemporary learning theory perspective on the etiology of anxiety disorders. *American Psychologist, 61*, 10-26.

Miserendino, M.J.D., Sananes, C.B., Melia, K.R., & Davis, M. (1990). Blocking of acquisition but not expression of conditioned fear-potentiated startle by NMDA antagonist in the amygdala. *Nature Letters, 345*, 716-718.

Mohanty, A., Gitelman, D.R., Small, D.M., & Mesulam, M.M. (2008). The spatial attention network interacts with limbic and moniaminergic systems to modulate motivation-induced attention shifts. *Cerebral Cortex, 18*, 2604-2613.

Mohr, C., Binkofski, F., Erdman, C., Büchel, C., & Helmchen, C. (2005). The anterior cingulate cortex contains distinct areas dissociating external from self-administered painful stimulation: a parametric fRMI study. *Pain, 114*, 347-357.

Moratti, S., Clementz, B.A., Gao, Y., Ortiz, T., & Keil, A. (2007). Neural mechanisms of evoked oscillations: stability and interaction with transient events. *Human Brain Mapping, 28*, 1318-1333.

Moratti, S., & Keil, A. (2005). Cortical activation during Pavlovian fear conditioning depends on hearth rate response patterns: an MEG study. *Cognitive Brain Research 25* 459-471.

Moratti, S., Keil, A., & Miller, G.A. (2006). Fear but not awareness predicts enhanced sensory processing in fear conditioning. *Psychophysiology, 43*, 216-226.

Moratti, S., Keil, A., & Stolarova, M. (2004). Motivated attention in emotional picture processing is reflected by activity modulation in cortical attention networks. *NeuroImage, 21*, 954-964.

Mucha, R.F., Pauli, P., & Weyers, P. (2006). Psychophysiology and implicit cognition in drug use: significance and measurement of motivation for drug use with emphasis on startle tests. In Wiers, R.W., Stacy & A.W. (Eds.), *Handbook of implicit cognition and addiction.* Thousand Oak, California.: Sage Publications, Inc.

Müller, M.M., & Hübner, R. (2002). Can the spotlight of attention be shaped like a doughnut? Evidence from steady-state visual evoked potentials. *Psychological Science, 13*, 119-124.

Müller, M. M., & Hillyard, S. (2000). Concurrent recording of steady-state and transient event-related potentials as indices of visual-spatial selective attention. *Clinical Neurophysiology, 111,* 1544-1552.

Naqvi, N.H., & Bechara, A. (2009). Tha hidden island of addiction: the insula. *Trend in Neurosciences, 32*, 56-67.

Neumann, D.L., & Waters, A.M. (2006). The use of the unpleasant sound as an unconditional stimulus in a human aversive Pavlovian conditioning procedure. *Biological Psychology 73*, 175-185.

O´Brien, C.P., Childress, A.R., Ehrman, R., & Robbins, S.J. (1998). Conditioning factors in drug abuse: can they explain compulsion? *Journal of Psychopharmacology, 12*, 12-22.

O´Doherty, J., Buchanan, T.W., Seymour, B., & Dolan, R.J. (2006). Predictive neural coding of reward preference involves dissociable responses in human ventral midbrain and ventral striatum. *Neuron, 49*, 157-166.

O´Doherty, J., Critchley, H., Deichmann, R., & Dolan, R.J. (2003). Dissociation valence of outcome from behavioral control in human orbital and ventral prefrontal cortices. *The Journal of Neuroscience, 23*, 7931-7939.

O´Doherty, J., Dayan, P., Schultz, J., Deichmann, R., Friston, K., & Dolan, R.J. (2004). Dissociable roles of ventral and dorsal striatum in instrumental conditioning. *Science, 304*, 452-454.

Öhman, A. (2005). The role of the amygdala inhuman fear: automatic detection of threat. *Psychoneuroendocrinology, 30*, 953-958.

Öhman, A., Carlsson, K., Lundqvist, D., & Ingvar, M. (2007). On the unconscious subcortical origin of human fear. *Physiology & Behaviur, 92*, 180-185.

Öhman, A., & Mineka, S. (2001). Fears, phobias, and preparedness: toward an evolved module of fear and fear learning. *Psychological Review, 108*, 483-522.

Oldfield, R.C. (1971). The assessment and analysis of handedness: The Edinburgh inventory. *Neuropsychologia, 9*, 97-113.

Packard, M.G., & McGaugh, J.L. (1996). Inactivation of hippocampus or caudate nucleus with lidocaine differently affects expression of place and response learning. *Neurobiology of Learning and Memory, 65*, 65-72.

Pardo, J.V., Fox, P.T., & Raichle, M.E. (1991). Localization of a human system for sustained attention by positron emossion tomography. *Nature, 349*, 61-64.

Parkinson, J.A., Olmstead, M.C., Burns, L.H., Robbins, T.W., & Everitt, B.J. (1999). Dissociation in effects of lesions of the nucleus accumbens core and shell on appetitive Pavlovian approach behavior and the potentiation of conditioned reinforcement and locomotor activity by $_{D}$-amphetamine. *The Journal of Neuroscience, 19*, 2401-2411.

Paulus, M.P. (2007). Decision-making dysfunctions in psychiatry altered homeostatic processing? *Science, 318*, 602-606.

Paulus, M.P., & Stein, M.B. (2006). An insular view of anxiety. *Biological Psychiatry, 60*, 383-387.

Pecina, S. (2008). Opioid reward "liking" and "wanting" in the nucleus accumbens. *Physiology & Behaviour, 94*, 675-680.

Pedrabissi, L., & Santinello, M. (1989). Inventario per l´ansia di "stato" e di "tratto". In Spielberger, C.D., Gorsuch, R.L., & Lushene R.E. (Eds.), *STAI, State- Trait Anxiety Inventory, Forma Y.* Firenze: O.S., Organizzazioni speciali.

Pessoa, L. (2005). To what extent are emotional visual stimuli processed without attention and awareness? *Current Opinion in Nuerobiology 15*, 188-196.

Phelps, A.E. (2006). Emotion and cognition: insignt from studies of the amygdala. *Annual Review Psychology, 57*, 27-53.

Phelps, E.A., Delgrado, M.R., Nearine, K.I., & LeDoux, J.E. (2004). Extinction learning in humans: role of the amygdala and vmPFC. *Neuron, 43*, 897-905.

Phelps, E.A., O´Connor, K.J., Gatenby, J.C., Gore, J.C., Grillon, C., & Davis, M. (2001). Activation of the left amygdala to a cognitive representation of fear. *Nature Neuroscience, 4,* 437-441.

Pizzagalli, D., Regard, M., & Lehmann, D. (1999). Rapid emotional face processing in the human right and left brain hemispheres: An ERP study. *NeuroReport, 10,* 2691-2698.

Ploghaus, A., Becerra, l., Borras, C., & Borsook, D. (2003). Neural circuit underlying pain modulation: expectation, hypnosis, placebo. *Trends in Cognitive Sciences, 7,* 197-200.

Ploghaus, A., Tracey, I., Gati, J.S., Clare, S., Menon, R.S., Matthews, P.M., & Rawlins, J.N.P. (1999). Dissociating pain from its anticipation in the human brain. *Science, 284,* 1979-1981.

Porro, C.A., Baraldi, P., Paragnoni, G., Serafini, M., Facchini, P., Maieron, M., & Nichelli, P. (2002). Does anticipation of pain affect cortical nociceptive system? *The Journal of Neuroscience, 22,* 3206-3214.

Price, J.L. (2003). Comparative aspects of amygdala connectivity. *Annals of the New York Academy of Science, 985,* 50-58.

Quirk, G.J., Garcia, R., & González-Lima, F. (2006). Prefrontal mechanisms in extinction of conditioned fear. *Biological Psychiatry, 60,* 337-343.

Redondo, J., & Marcos, J.L. (2003). Effects of CS-US interval on unconditioned response diminution in human heart rate classical conditioning. *Journal of Psychophysiology, 17,* 30-38.

Regan, D. (1989). *Huamn brain electrophysiology: Evoked potentials and evoked magnetic fields in science and medicine.* New York: Elsevier.

Reiff, S., Katkin, E.S., & Friedman, R. (1999). Classical conditioning of the human blood pressure response. *International Journal of Psychophysiology, 34,* 135-145.

Rescorla, R.A. (1968). Probability of shock in the presence and absence of CS in fear conditioning. *Journal of Comparative and Physiological Psychology, 66,* 1-5.

Rescorla, R.A. (1988). Pavlovian conditioning. It´s not what you think it is. *American Psychologist, 43,* 151-160.

Rescorla, R.A., & Solomon, R.L. (1967). Two-process learning theory: relationships between pavlovian conditioning and instrumental learning. *Psychological Review, 74,* 151-182.

Rogan, M.T., Leon, K.S., Perez, D.L., & Kandel, E.R. (2005). Distinct neural signatures for safety and danger in the amygdala and striatum of the mouse. *Neuron, 46,* 309-320.

Rolls, E.T. (2000). The orbital cortex and reward. *Cerebral Cortex, 10,* 284-294.

Romaniuk, C.B., & Williams, D.A. (2000). Conditioning across the duration of a backward conditioned stimulus. *Journal of Experimental Psychology: Animal Behavior Processes, 26*, 454-461.

Rothman, R.B., Blough, B.E., & Baumann, M.H. (2008). Dual dopamine/serotonin releasers: potential treatment agents for stimulant addiction. *Experimental and Clinical Psychopharmacology, 16*, 458-474.

Salvy, S.J., Pierce, W.D., Heth, D.C., & Russell, J.C. (2004). Taste avoidance induced by wheel running: effects of backward pairings and robustness of conditioned taste aversion. *Physiology & Behaviour, 82*, 303-308.

Schiller, D., Levy, I., Niv, Y., LeDoux, J.E., & Phelps, E.A. (2008). From fear to safety and back: reversal of fear in the human brain. *The Journal of Neuroscience, 28*, 11517-11525.

Schiller, D., Monfils, M.H., Raio, C.M., Johnson, D.C., LeDoux, J.E., & Phelps, A.E. (2009). Preventing the return of fear in humans using reconsolidation update mechanisms. *Nature, 462*, 1-6.

Schmidt, A., Koch, M., & Schnitzler, H.U. (1995). Conditioned pleasure attenuates the startle response in rats. *Neurobiology of Learning and Memory, 64*, 1-3.

Schneider, M., & Spanagel, R. (2008). Appetitive odor-cue conditioning attenuates the acoustic startle response in rats. *Behavioral Brain Research, 189*, 226-230.

Schultz, W. (2007). Behavioral dopamine signlas. *TRENDS in Neurosciences, 30*, 203-210.

Schultz, W., Dayan, P., & Montague, P.R. (1997). A neural substrate of prediction and reward. *Science, 275*, 1593-1599.

Schultz, W., Tremblay, L., & Hollerman, J.R. (2003). Changes in behavior-related neuronal activity in the striatum during learning. *TRENS in Neurosciences, 26*, 321-328.

Schupp, H.T., Junghöfer, M., Weike, A.I., & Hamm, A.O. (2003). Emotional facilitation of sensory processing in the visual cortex. *Psychological Science, 14*, 7-13.

Seligman, M.E.P., & Binik, Y.M. (1971). The safety signal hypothesis. In Davis, H., Hurwitz & H.M.B. (Eds.), *Operant-Pavlovian Interactions*: Lawrence Erlbaum Associates, Publishers.

Seymour, B., Daw, N., Dayan, P., Singer, T., & Dolan, R. (2007). Differential encoding of losses and gains in the human striatum. *The Journal of Neuroscience, 27*, 4826-4831.

Seymour, B., O´Doherty, J.P., Koltzenburg, M., Wiech, K., Frackowiak, R., Friston, K., & Dolan, R. (2005). Opponent appetitive-aversive neural processes underlie predictive learning of pain relief. *Nature, 8*, 1234-1240.

Shibata, H., Kondo, S., & Naito, J. (2004). Organisation of retrosplenial cortical projections to the anterior cigulate, motor, and prefrontal cortices in the rat. *Neuroscience Research, 49*, 1-11.

Silberstein, R.C., Ciorciari, J., & Pipingas, A. (1995). Steady-state visually evoked potential topography during the Wisconsin card sorting test. *Electroencephalography and clinical Neurophysiology, 96*, 24-35.

Skrandies, W., & Jedynak, A. (2000). Associative learning in humans - conditioning of sensory-evoked brain activity. *Behavioral Brain Research, 107*, 1-8.

Small, D.M., Zatorre, R.J., Dagher, A., Evans, A.C., & Jones-Gotman, M. (2001). Changes in brain activity related to eating chocolate. *Brain, 124*, 1720-1733.

Solomon, R.L. (1980). The opponent-process theory of acquired motivation. The cost of pleasure and the benefitd of pain. *American Psychologist, 35*, 691-712.

Solomon, R.L., & Corbit, J.D. (1974). An opponent process theory of motivation: temporal dynamics of affect. *Psychological Review, 81*, 119-145.

Spetch, M.L., Wilkie, D.M., & Pinel, J.P. (1981). Backward conditioning: a reevaluation of the empirical evidence. *Psychological Bullettin, 89*, 163-175.

Spielberger, C.D., Gorsuch, R.L., & Lushene, R.E. (1996). STAI. In C. I. P. Scalarum (Ed.), *Internationale Skalen für Psychiatrie* (4 ed.). Göttingen: Beltz-Test.

Squire, L.R., & Zola-Morgan, S. (1991). The medial temporal lobe memory system. *Science, 253*, 1380-1386.

Strack, F., & Deutsch, R. (2004). Reflective and impulsive determinants of social behavior. *Personality and Social Psychology Review, 8*, 220-247.

Tabbert, K., Stark, R., Kirsch, P., & Vaitl, D. (2005). Hemodynamic responses of the amygdala, the orbitofrontal cortex and the visual cortex during a fear conditioning paradigm. *International Journal of Psychophysiology, 57*, 15-23.

Tabbert, K., Stark, R., Kirsch, P., & Vaitl, D. (2006). Dissociation of neural responses and skin conductance reactions during fear conditioning with and without awareness of stimulus contingencies. *NeuroImage, 32*, 761-770.

Tabuchi, E., Furusawa, A.A., Hori, E., Umeno, K., Ono, T., & Nishijo, H. (2005). Neural correlates to action and rewards in the rat posterior cingulate cortex. *NeuroReport, 16*, 949-953.

Tanimoto, H., Heisenberg, M., & Gerber, B. (2004). Event timing turns punishment to reward. *Nature, 430*, 983.

Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., Mazoyer, B., & Joliot, M. (2002). Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *NeuroImage, 15*, 273-289.

Ungless, M.A. (2004). Dopamine: the salient issue. *Trend in Neurosciences, 27*, 702-706.

Urushihara, K. (2004). Excitatory backward conditioning in an appetitive conditioned reinforcement preparation with rats. *Behavioral Processes, 67*, 477-489.

Vogel, E.H., Castro, M.E., & Saavedra, M.A. (2004). Quantitative models of Pavlovian conditioning. *Brain Resesearch Bullettin, 63*, 173-202.

Vogt, B.A., Nimchinsky, E.A., Vogt, L.J., & Hof, P.R. (1995). Human cingulate cortex: surface features, flat maps, and cytoarchitecture. *The Journal of Comparative Neurology, 359*, 490-506.

Wagner, A.R. (1981). SOP: A model of automatic memory processing in animal behavior. In Spear, N.E, Miller & R.R. (Eds.), *Information processing in animals: Memory mechanisms.* (pp. 5-47). Hillsdale: NJ: Erlbaum.

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

Weiss, F. (2005). Neurobiology of craving, conditioned reward and relapse. *Current Opinion in Pharmacology, 5*, 9-19.

Wise, R.A. (2004). Dopamine, learning and motivation. *Nature Reviews Neuroscience, 5*, 483-494.

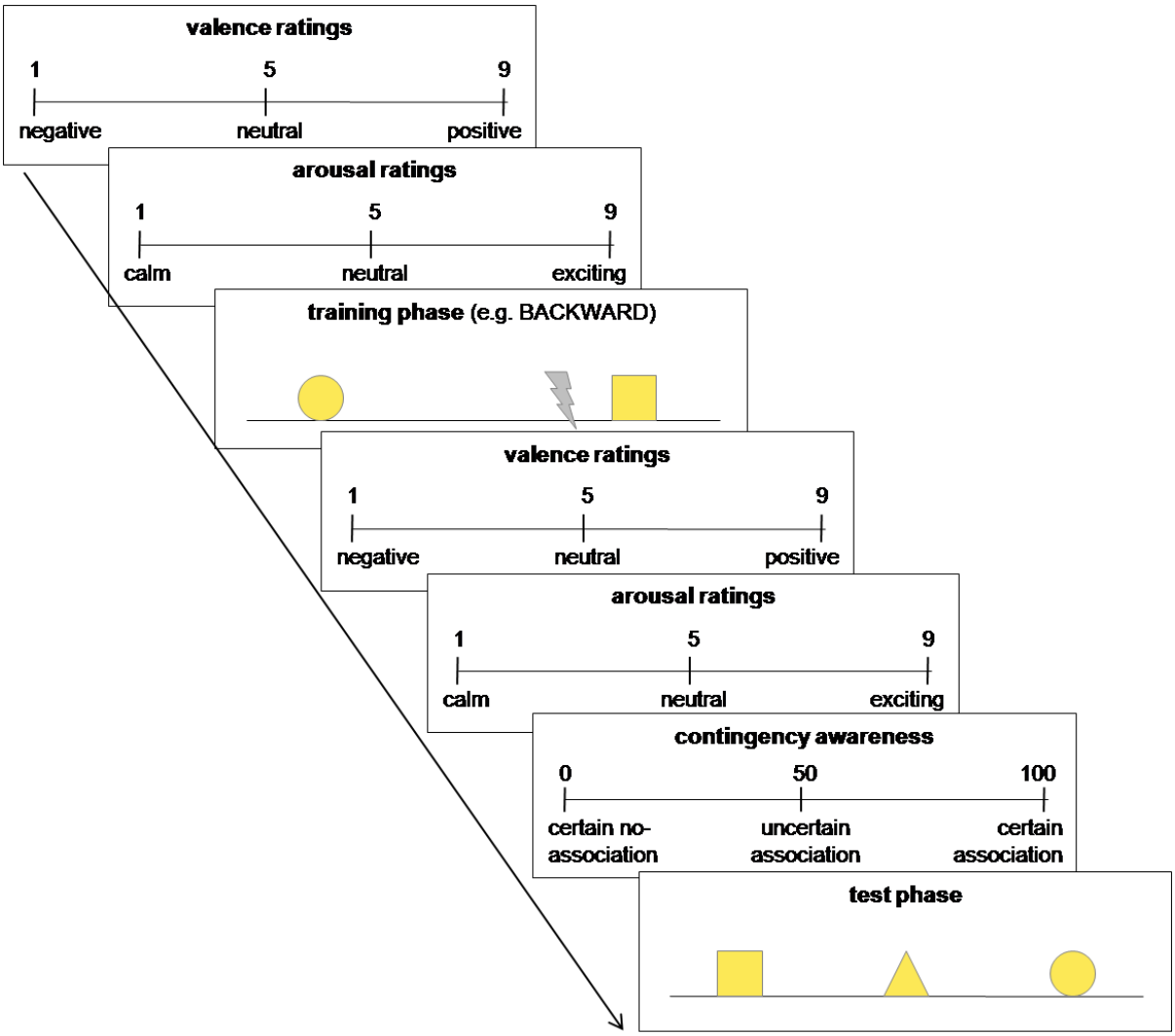
Yarali, A., Ehser, S., Hapil, F.Z., Huang, J., & Gerber, B. (2009). Odour intensitiy learning in fruit flies. *Proceedings of the Royal Society B, 276*, 3414-3420.

Yarali, A., Krischke, M., Michels, B., Saumweber, T., Mueller, M.J., & Gerber, B. (2008). Genetic distortion of the balance between punishment and relief learning in *Drosophila*. *Journal of Neurogenetic, 23*, 235-247.

Yin, H.H., & Knowlton, B.J. (2006). The role of the basal ganglia in habit formation. *Nature Reviews Neuroscience, 7*, 464-476.

# 8. Appendix

## 8.1. Sketch of the Paradigm



## 8.2. Informed Consent Form

### *8.2.1. Informed Consent Form for the Study 1*

**Aufklärungstext zur Studie**

**„Erlernte Vermeidung und Annäherung durch schmerzhafte Stimuli: Physiologische Korrelate“**

Sehr geehrter Versuchsteilnehmer, sehr geehrte Versuchsteilnehmerin,

Sie nehmen an einer Studie teil, bei der wir untersuchen möchten, wie Schmerzreize Ihre Herzrate und Ihre Muskelspannung verändern. Sie werden aus der Teilnahme keinen unmittelbaren Nutzen für sich ziehen können. Wir hoffen jedoch, durch unsere Arbeit mehr darüber erfahren zu können, welche unmittelbaren physiologischen Reaktionen und welche gefühlsmäßigen und motivationalen Veränderungen schmerzhafte Reize hervorrufen können. Wenn Sie möchten, werden wir Ihnen nach der Untersuchung gerne die Hintergründe und Ziele dieser Untersuchung ausführlich schildern.

Vor der Untersuchung werden Sie einige Fragebögen ausfüllen, in denen wichtige Daten bezüglich Ihrer Person festgehalten werden. Dann wird die Versuchsleiterin zur Messung Ihrer Herzrate und Ihrer Muskelspannung insgesamt sechs Messelektroden in Ihrem Gesicht und auf Ihrer Brust anbringen. Dazu wird sie Ihre Haut mit etwas Alkohol reinigen, damit der Widerstand zwischen Haut und Messelektrode so gering wie möglich ist. Aufgrund dieser Hautreinigung kann es zu Hautrötungen und leichten Hautirritationen kommen, die aber normalerweise innerhalb kurzer Zeit abklingen.

Im ersten Teil der Untersuchung werden Sie auf einem Computerbildschirm geometrische Figuren sehen, dabei werden Sie manchmal elektrische Reize am Unterarm verspüren. Die elektrischen Reize sind etwas schmerzhaft, aber sehr kurz.

Im zweiten Teil der Untersuchung werden Sie die geometrischen Figuren aus dem ersten Teil der Untersuchung noch mal zu sehen bekommen. Dabei werden Sie über Kopfhörer manchmal ein kurzes Geräusch hören. Dieses Geräusch kann etwas unangenehm für Sie sein, ist aber vollkommen unschädlich.

Damit Sie sich den Untersuchungsablauf vorstellen können, zeigen wir Ihnen zu Beginn einige Beispielfiguren, und auch Beispiele für die elektrischen Reize und die Geräusche werden Sie erhalten. Die Stärke der elektrischen Reize wird individuell ermittelt und vor Versuchsbeginn festgelegt.

**Die Teilnahme an der Untersuchung ist völlig freiwillig. Sie können jederzeit - ohne Angabe von Gründen - die Teilnahme abbrechen. Dadurch entstehen Ihnen keinerlei persönliche Nachteile. Für Ihre Teilnahme an der Untersuchung erhalten Sie 2 Versuchspersonsstunden.**

Alle Daten dienen ausschließlich Forschungszwecken, werden vertraulich behandelt und ohne Namensgebung unter einer Codenummer abgespeichert. Die Videoaufzeichnungen werden nach der statistischen Datenauswertung gelöscht, alle anderen Daten werden für unbestimmte Zeit gespeichert. Der Codierungsschlüssel wird nach Abschluss der Studie vernichtet. Bis dahin können Sie auch noch nach der Untersuchung die Löschung ihrer Daten verlangen.

Falls Sie noch weitere Frage haben, fragen Sie bitte jetzt.

## Einverständniserklärung

Ich bin einverstanden, an dem Experiment „Erlernte Vermeidung und Annäherung durch schmerzhafte Stimuli: Physiologische Korrelate“ teilzunehmen und dass die erhobenen Daten in anonymisierter Form wissenschaftlich ausgewertet werden.

Ich bin darüber informiert worden, dass ich jederzeit aus der Untersuchung ausscheiden kann, ohne dass mir persönliche Nachteile entstehen.

Mit meiner Unterschrift erkläre ich, dass ich das Vorhaben und diese Information verstanden habe, meine Fragen zufrieden stellend beantwortet wurden und ich freiwillig und aus eigenem Entschluss an der Untersuchung teilnehme.

Würzburg, den _____ Unterschrift

_____

Name und Anschrift _____

Unterschrift des Versuchsleiters _____ Code_____

## *8.2.2. Informed Consent Form for the Study 2*

Julius-Maximilians-
UNIVERSITÄT
WÜRZBURG

Lehrstuhl für Psychologie I - Prof. Dr. Paul Pauli
Biologische Psychologie, Klinische Psychologie und Psychotherapie

### Informationsblatt

### Funktionelle Kernspintomographie

***Titel der Studie: Konditionierte Vermeidung und Annäherung durch schmerzhafte Stimuli bei Menschen***

### Sehr geehrte(r) Proband(in),

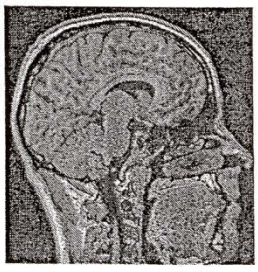
die Kernspintomographie benutzt anstelle von Röntgenstrahlen oder radioaktiven Kontrastmitteln Radiowellen zur Abbildung des Gehirns und seiner Funktionen. Dazu ist es notwendig, dass Sie sich innerhalb des Magnetfeldes des Kernspintomographen befinden. Ihr Kopf liegt dabei in einer speziellen Kopfspule, die sie nicht belästigt oder drückt. Die von der Kopfspuhle empfangenen Signale werden im Computer weiterverarbeitet und können so zur Erstellung von Bildern verwandt werden. Diese Technik wird weltweit eingesetzt. Es sind bislang keine schädigenden Wirkungen aufgetreten. Es werden **keine** Kontrastmittel gespritzt.

### Untersuchungsablauf

Die Untersuchung wird mit einem modernen 1,5- Tesla Kernspintomographen durchgeführt. Sie liegen dabei auf einer Liege, die in das Magnetfeld hineingefahren wird. Bei der Untersuchung treten Klopfgeräusche, die auf elektromagnetischen Schaltvorgängen im Magneten beruhen. Während der Messung sollten sie ruhig und entspannt liegen, insbesondere sollte sich der Kopf nicht bewegen. Die Untersuchung im Tomographen dauert zwischen 30 und 60 Minuten. Während der Untersuchung werden sie optisch (über eine Kamera) und akustisch (über Lautsprecher und Mikrophon) überwacht und erhalten einen Alarmknopf in die Hand, so dass die Untersuchung, im Bedarfsfall **jederzeit** abgebrochen werden kann.

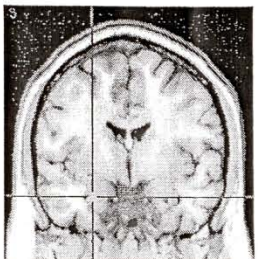
### Geplante Untersuchungen:

**Anatomie/Volumetrie:**



Die bei Ihnen geplante Untersuchung ermöglicht die bildliche Darstellung und/oder Vermessung des Gehirns

**Funktionelle Kernspintomographie:**



Die bei Ihnen geplante Untersuchung ermöglicht die bildliche Darstellung von funktionellen Zentren des Gehirns. Hierzu werden Bildserien in Ruhe und während der Ausführung einer Aktivierungsaufgabe (z.B. Betrachten von Bildern) aufgenommen. Die Aktivierungsaufgabe wird Ihnen vor der Untersuchung ausführlich erläutert.

In der Studie geht es darum, die Wirkung von Schmerz auf die Verarbeitung visueller Reize im Gehirn zu untersuchen. Wir hoffen, durch unsere Arbeit mehr darüber erfahren zu können, wie Schmerz die Verarbeitung visueller Objekte im Gehirn beeinflusst und welche gefühlsmäßigen und motivationalen Veränderungen schmerzhafte Reize hervorrufen können. In einem funktionellen Magnet-Resonanz-Tomographen (= Scanner) werden ihnen geometrische Figuren gezeigt, dabei werden Sie manchmal elektrische Reize am Unterarm verspüren. Die elektrischen Reize sind vollkommen ungefährlich, sollen aber leicht schmerzhaft sein. Die Stärke der elektrischen Reize wird individuell ermittelt und vor Versuchsbeginn festgelegt. Während des Experimentes wird die Aktivität Ihres Gehirns gemessen. Vor und nach der Untersuchung im Scanner werden sie gebeten einige Fragebögen auszufüllen. Der Ablauf der gesamten Untersuchung dauert ca. 1 Stunde.

Im Scanner werden folgende Messungen durchgeführt:

- Kurze Messung der Position des Kopfes im Scanner ( ca. 6 sec.)
- Anatomische Messung (ca. 8 min)
- Bilder Studie (ca. 40 min)

Notwendige Instruktionen erhalten sie zu Beginn über den Bildschirm. Zusätzlich wird sie der/die Versuchsleiter/in zwischen den einzelnen Messungen über Lautsprecher kontaktieren. Scheuen Sie sich nicht Fragen zu stellen, falls etwas unklar geblieben ist.

Als Aufwandsentschädigung werden Ihnen die entsprechenden Versuchspersonenstunden attestiert und/oder eine CD zur anatomischen Messung ausgegeben werden.

Selbstverständlich werden Ihre Daten streng anonym behandelt. Die Zuordnung Ihrer Kennnummer zu den Daten ist nur für die Studienleiter und die Versuchsleiter/innen möglich. Ihre Daten werden zunächst auf unbestimmte Zeit zur weiteren Auswertung aufbewahrt. Sollten Sie aber eine Löschung Ihrer Daten wünschen, ist dies zu jedem Zeitpunkt möglich.

Mit der Einverständniserklärung (s. Anhang) bestätigen Sie, dass Sie über die bevorstehende Untersuchung informiert wurden.

**Vielen Dank für Ihre Mitarbeit!**

Zur anonymisierten Zuweisung der Fragebögen geben Sie bitte hier Ihren Code an, bestehend aus dem Geburtsdatum ihrer Mutter und Ihres Vaters:

Geburtsd. Mutter (TT.MM): _____ Geburtsd. Vater (TT.MM): _____

Ich bin darüber informiert worden, dass ich die Untersuchung jederzeit abbrechen kann, ohne dass mir persönlich Nachteile entstehen.

Ich bin damit einverstanden, dass die erhobenen Daten zu Forschungszwecken anonym gespeichert werden.

Würzburg, den:_____ Unterschrift:_____

Geburtsdatum:_____

Name und Anschrift:

_____

_____

_____

_____

Unterschrift des Untersuchungsleiters:_____

*8.2.3. Informed Consent Form for the Study 3*

**Consenso informato per la ricerca**

**“Condotte di evitamento e di avvicinamento condizionati da stimoli dolorosi. Uno studio con potenziali evocati”.**

Gentile partecipante,

Nello studio a cui sta per partecipare, desideriamo approfondire l´elaborazione cognitiva degli stimoli dolorosi nell´uomo. Con tale lavoro, noi speriamo di fornire maggiori informazioni sulle risposte corticali indotte da uno stimolo aversivo. Se lo desidera, alla fine della ricerca potremo spiegarle in modo più dettagliato sia lo scopo che il contesto teorico dello studio.

Per prima cosa, dovrà compilare alcuni questionari. Sappia però che ogni informazione riguardante la sua persona, inclusa la sua identità, saranno mantenute riservate e usate solo per gli scopi dello studio.

Lo sperimentatore poi Le applicherà una cuffia elastica all´interno della quale sono fissati diversi sensori di superficie al fine di misurare la sua attività elettroencefalografica. L’inserimento di un gel all’interno di ciascun sensore faciliterà la trasmissione del segnale da Lei prodotto. La procedura di applicazione di tali sensori è piuttosto lunga, ma non è assolutamente dolorosa.

Sul suo avambraccio sinistro saranno inoltre applicati due sensori per la somministrazione di una leggera scossa elettrica. L´intensità della scossa che sarà usata durante lo studio sarà testata prima dell´inizio della sessione sperimentale e potrà decidere se proseguire o se rinunciare.

**Ricorda che la sua partecipazione è assolutamente volontaria, potrà quindi cambiare idea e ritirarsi dall´esperimento in qualsiasi momento senza dare alcuna giustificazione.**

Nella prima sessione dell´esperimento vedrà una serie di figure geometriche sullo sfondo nero di uno schermo del computer. Talvolta percepirà delle scosse elettriche, le quali saranno lievemente dolorose, ma molto brevi. Nella seconda sessione della ricerca rivedrà le figure geometriche della prima sessione e inoltre una nuova figura. Il tuo compito sarà di osservare le figure cercando di muoversi il meno possibile.

Lo studio durerà circa 2 ore e per la sua partecipazione riceverà un compenso di 12 €.

**Consenso informato**

Io _____ acconsento di partecipare allo studio: "Il comportamento di evitamento e di avvicinamento indotti da stimoli dolorosi. Uno studio con potenziali evocati" e all´analisi dei miei dati personali per scopi sperimentali.

Sono stato inoltre informato della possibilità di interrompere lo studio in ogni momento.

Con la mia firma qui sotto dichiaro di aver compreso e accettato le condizioni dello studio, di essere soddisfatto delle risposte fornitemi alle mie domande e di partecipare volontariamente alla ricerca.

Padova, _____ Firma _____

Nome e indirizzo _____

_____

Firma del ricercatore _____ Codice_____

## 8.3. Questionnaires

### *8.3.1. Experiment Sign-up Sheet, Study 1 and Study 2*

**Versuchspersonenprotokoll**

Datum:_____ Versuchsleiter: _____

Vp-Code: _____

**Angaben zur Person:**

Alter: _____ Jahre Geschlecht: □ männlich □ weiblich

Beruf und/oder Studienfach:

_____

Muttersprache:

_____

Linkshänder ☐ Rechtshänder ☐

Neurologische Erkrankungen bekannt?

_____

Substanzeinnahme (Medikamente, Drogen, Alkohol) in letzter Woche: □ nein □ ja

falls ja, wann erfolgte die letzte Einnahme?

_____

falls ja, welche Substanzen?_____

momentan Schmerzen? □ nein □ ja

Wenn ja, wo?_____

Notizen/Sonstiges:

_____

_____

*8.3.2. Experiment Sign-up Sheet Study 3*

**SCHEDA SOGGETTO**

Data:_____

Codice soggetto:_____

Nome File:_____

---

Nome e cognome:_____

Età:_____

Sesso: M F

Scolarità: _____

Professione: _____

Manualità: _____

Occhiali/Lenti: Sì No

Disturbi neurologici/ Traumi ecc.:_____

_____

Eventuali farmaci assunti:_____

_____

Hai bevuto caffé tè o alcool ieri sera?_____

_____

Hai bevuto caffé tè o alcool prima di venire in laboratorio?_____

_____

Eventi fisici rilevanti?_____

_____

Eventi psicologici rilevanti? _____

_____

Fuma? SI NO

Se sì, quanto?_____

Ha fumato prima di venire in laboratorio? SI NO

Commenti:

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

### *8.3.3. STAI, German Version*

Heutiges Datum: _____ Vp-Nr.: _____ Geschlecht: m / w

Versuchsbedingung _____

**STAI-State**

| **Anleitung:** Im folgenden Fragebogen finden Sie eine Reihe von Feststellungen, mit denen man sich selbst beschreiben kann. Bitte lesen Sie jede Feststellung durch und wählen Sie aus den vier Antworten diejenige aus, die angibt, wie Sie sich **jetzt**, d. h. **in diesem Augenblick** fühlen. Kreuzen Sie bitte bei jeder Feststellung die Zahl unter der von Ihnen gewählten Antwort an. Es gibt keine richtigen oder falschen Antworten. Überlegen Sie bitte nicht lange und denken sie daran, diejenige Antwort auszuwählen, die Ihren **augenblicklichen** Gefühlszustand am besten beschreibt. | *Überhaupt nicht* | *Ein wenig* | *ziemlich* | *sehr* |
|---|---|---|---|---|
| 1. Ich bin ruhig | *1* | *2* | *3* | *4* |
| 2. Ich fühle mich geborgen | *1* | *2* | *3* | *4* |
| 3. Ich fühle mich angespannt | *1* | *2* | *3* | *4* |
| 4. Ich bin bekümmert | *1* | *2* | *3* | *4* |
| 5. Ich bin gelöst | *1* | *2* | *3* | *4* |
| 6. Ich bin aufgeregt | *1* | *2* | *3* | *4* |
| 7. Ich bin besorgt, dass etwas schief gehen könnte | *1* | *2* | *3* | *4* |
| 8. Ich fühle mich ausgeruht | *1* | *2* | *3* | *4* |
| 9. Ich bin beunruhigt | *1* | *2* | *3* | *4* |
| 10. Ich fühle mich wohl | *1* | *2* | *3* | *4* |
| 11. Ich fühle mich selbstsicher | *1* | *2* | *3* | *4* |
| 12. Ich bin nervös | *1* | *2* | *3* | *4* |
| 13. Ich bin zappelig | *1* | *2* | *3* | *4* |
| 14. Ich bin verkrampft | *1* | *2* | *3* | *4* |
| 15. Ich bin entspannt | *1* | *2* | *3* | *4* |
| 16. Ich bin zufrieden | *1* | *2* | *3* | *4* |
| 17. Ich bin besorgt | *1* | *2* | *3* | *4* |
| 18. Ich bin überreizt | *1* | *2* | *3* | *4* |
| 19. Ich bin froh | *1* | *2* | *3* | *4* |
| 20. Ich bin vergnügt | *1* | *2* | *3* | *4* |

Heutiges Datum: _____ Vp-Nr.: _____ Geschlecht: m / w

Versuchsbedingung _____

## STAI-Trait

| **Anleitung:** Im folgenden Fragebogen finden Sie eine Reihe von Feststellungen, mit denen man sich selbst beschreiben kann. Bitte lesen Sie jede Feststellung durch und wählen Sie aus den vier Antworten diejenige aus, die angibt, wie Sie sich **im allgemeinen** fühlen. Kreuzen Sie bitte bei jeder Feststellung die Zahl unter der von Ihnen gewählten Antwort an.<br>Es gibt keine richtigen oder falschen Antworten. Überlegen Sie bitte nicht lange und denken Sie daran, diejenige Antwort auszuwählen, die am besten beschreibt, wie Sie sich im **allgemeinen** fühlen. | *Fast nie* | *Manchmal* | *Oft* | *Fast immer* |
|---|---|---|---|---|
| 21. Ich bin vergnügt | *1* | *2* | *3* | *4* |
| 22. Ich werde schnell müde | *1* | *2* | *3* | *4* |
| 23. Mir ist zum Weinen zumute | *1* | *2* | *3* | *4* |
| 24. Ich glaube, mir geht es schlechter als anderen Leuten | *1* | *2* | *3* | *4* |
| 25. Ich verpasse günstige Gelegenheiten, weil ich mich nicht schnell genug entscheiden kann | *1* | *2* | *3* | *4* |
| 26. Ich fühle mich ausgeruht | *1* | *2* | *3* | *4* |
| 27. Ich bin ruhig und gelassen | *1* | *2* | *3* | *4* |
| 28. Ich glaube, dass mir meine Schwierigkeiten über den Kopf wachsen | *1* | *2* | *3* | *4* |
| 29. Ich mache mir zuviel Gedanken über unwichtige Dinge | *1* | *2* | *3* | *4* |
| 30. Ich bin glücklich | *1* | *2* | *3* | *4* |
| 31. Ich neige dazu, alles schwer zu nehmen | *1* | *2* | *3* | *4* |
| 32. Mir fehlt es an Selbstvertrauen | *1* | *2* | *3* | *4* |
| 33. Ich fühle mich geborgen | *1* | *2* | *3* | *4* |
| 34. Ich mache mir Sorgen über mögliches Missgeschick | *1* | *2* | *3* | *4* |
| 35. Ich fühle mich niedergeschlagen | *1* | *2* | *3* | *4* |
| 36. Ich bin zufrieden | *1* | *2* | *3* | *4* |
| 37. Unwichtige Gedanken gehen mir durch den Kopf und bedrücken mich | *1* | *2* | *3* | *4* |
| 38. Enttäuschungen nehme ich so schwer, dass ich sie nicht vergessen kann | *1* | *2* | *3* | *4* |
| 39. Ich bin ausgeglichen | *1* | *2* | *3* | *4* |
| 40. Ich werde nervös und unruhig, wenn ich an meine derzeitigen Angelegenheiten denke | *1* | *2* | *3* | *4* |

*8.3.4. STAI, Italian Version*

**STAI-Y1 (STAI State)**

Spielberg 1988

ISTRUZIONI

Qui di seguito sono riportate alcune frasi che le persone spesso usano per descriversi. Legga ciascuna frase e poi segni con una crocetta come si sente ***ADESSO***, cioè in questo momento. Non ci sono risposte giuste o sbagliate. Non impieghi troppo tempo per rispondere alle domande e dia la risposta che le sembra descrivere meglio i suoi *attuali* stati d'animo.

| | | Per nulla | Un po' | Abbastanza | Moltissimo |
|---|---|---|---|---|---|
| 1. | Mi sento calmo……..……..……. | 1 | 2 | 3 | 4 |
| 2. | Mi sento sicuro…….……. | 1 | 2 | 3 | 4 |
| 3. | Sono teso……...……... | 1 | 2 | 3 | 4 |
| 4. | Mi sento sotto pressione……...…… | 1 | 2 | 3 | 4 |
| 5. | Mi sento tranquillo…….……..…….. | 1 | 2 | 3 | 4 |
| 6. | Mi sento turbato…….……... | 1 | 2 | 3 | 4 |
| 7. | Sono attualmente preoccupato per possibili disgrazie | 1 | 2 | 3 | 4 |
| 8. | Mi sento soddisfatto…….…. | 1 | 2 | 3 | 4 |
| 9. | Mi sento intimorito…….……..…..… | 1 | 2 | 3 | 4 |
| 10. | Mi sento a mio agio..……..…..…. | 1 | 2 | 3 | 4 |

| | | | | | |
|---|---|---|---|---|---|
| 11. | Mi sento sicuro di me……..… | 1 | 2 | 3 | 4 |
| 12. | Mi sento nervoso……..... | 1 | 2 | 3 | 4 |
| 13. | Sono agitato…..……..…… | 1 | 2 | 3 | 4 |
| 14. | Mi sento indeciso…….……...…. | 1 | 2 | 3 | 4 |
| 15. | Sono rilassato……...……..…….…. | 1 | 2 | 3 | 4 |
| 16. | Mi sento contento…...……..… | 1 | 2 | 3 | 4 |
| 17. | Sono preoccupato……..…. | 1 | 2 | 3 | 4 |
| 18. | Mi sento confuso…..……. | 1 | 2 | 3 | 4 |
| 19. | Mi sento disteso……..……. | 1 | 2 | 3 | 4 |
| 20. | Mi sento bene..…… | 1 | 2 | 3 | 4 |

**STAI-Y2 (STAI Trait)**

Spielberg 1988

ISTRUZIONI

Qui di seguito sono riportate alcune frasi che le persone spesso usano per descriversi. Legga ciascuna frase e poi segni con una crocetta come ***ABITUALMENTE*** si sente. Non ci sono risposte giuste o sbagliate. Non impieghi troppo tempo per rispondere alle domande e dia la risposta che le sembra descrivere meglio come lei ***ABITUALMENTE*** si sente.

| | | Per nulla | Un po’ | Abbastanza | Moltissimo |
|---|---|---|---|---|---|
| 1. | Mi sento bene……. | 1 | 2 | 3 | 4 |
| 2. | Mi sento teso e irrequieto…….……. | 1 | 2 | 3 | 4 |
| 3. | Sono soddisfatto di me stesso……. | 1 | 2 | 3 | 4 |
| 4. | Vorrei poter essere felice come sembrano essere gli altri. | 1 | 2 | 3 | 4 |
| 5. | Mi sento un fallito…….. | 1 | 2 | 3 | 4 |
| 6. | Mi sento riposato……... | 1 | 2 | 3 | 4 |
| 7. | Io sono calmo, tranquillo e padrone di me…… | 1 | 2 | 3 | 4 |
| 8. | Sento che le difficoltà si accumulano tanto da non poterle superare | 1 | 2 | 3 | 4 |
| 9. | Mi preoccupo troppo di cose che in realtà non hanno importanza | 1 | 2 | 3 | 4 |
| 10. | Sono felice……. | 1 | 2 | 3 | 4 |
| 11. | Mi vengono pensieri negativi…… | 1 | 2 | 3 | 4 |

| | | | | | |
|---|---|---|---|---|---|
| 12. | Manco di fiducia in me stesso…….. | 1 | 2 | 3 | 4 |
| 13. | Mi sento sicuro……..…….…. | 1 | 2 | 3 | 4 |
| 14. | Prendo decisioni facilmente…….…. | 1 | 2 | 3 | 4 |
| 15. | Mi sento inadeguato…….…. | 1 | 2 | 3 | 4 |
| 16. | Sono contento…… | 1 | 2 | 3 | 4 |
| 17. | Pensieri di scarsa importanza mi passano per la mente e mi infastidiscono | 1 | 2 | 3 | 4 |
| 18. | Vivo le delusioni con tanta partecipazione da non poter togliermele dalla testa | 1 | 2 | 3 | 4 |
| 19. | Sono una persona costante……. | 1 | 2 | 3 | 4 |
| 20. | Divento teso e turbato quando penso alle mie attuali preoccupazioni | 1 | 2 | 3 | 4 |

### *8.3.5. The Edinburg Inventory (Italian)*

**EDINBURGH HANDEDNESS INVENTORY (Oldfield, 1971)**

Metti una crocetta sul numero appropriato nella tabella qui rappresentata per indicare quale mano preferisci usare per ciascuna delle attività indicate.

Se la tua preferenza per una mano è così forte che non proveresti mai ad usare l'altra se non assolutamente costretto\a, metti una crocetta su "**-2**" o "**2**" (a seconda della mano). Se preferisci una mano all'altra in modo meno categorico, metti una crocetta su "**-1**" o "**1**" (a seconda della mano). Se per te è realmente indifferente usare l'una o l'altra mano, metti una crocetta sullo "**0**".

Alcune delle attività descritte richiedono entrambe le mani. In questi casi, il compito, o l'oggetto, per cui è richiesta la preferenza è indicato in parentesi.

Per favore cerca di rispondere a tutte le domande e lasciale in bianco solo se non hai mai avuto alcuna esperienza dell'attività indicata.

| **Attività** | Mano Preferita | | | | |
|---|---|---|---|---|---|
| | **Sinistra** | | | | **Destra** |
| Scrivere | -2 | -1 | 0 | 1 | 2 |
| Disegnare | -2 | -1 | 0 | 1 | 2 |
| Lanciare un oggetto | -2 | -1 | 0 | 1 | 2 |
| Usare le forbici | -2 | -1 | 0 | 1 | 2 |
| Usare lo spazzolino da denti | -2 | -1 | 0 | 1 | 2 |
| Usare il coltello senza forchetta | -2 | -1 | 0 | 1 | 2 |
| Usare il cucchiaio | -2 | -1 | 0 | 1 | 2 |
| Impugnare la scopa (mano più in alto) | -2 | -1 | 0 | 1 | 2 |
| Accendere un fiammifero | -2 | -1 | 0 | 1 | 2 |
| Aprire una scatola (coperchio) | -2 | -1 | 0 | 1 | 2 |

## 8.4. Pain Threshold Procedure

*8.4.1. Sheet for the Pain Threshold for the Study 1 and the Study 2*

Vp. Code: _____

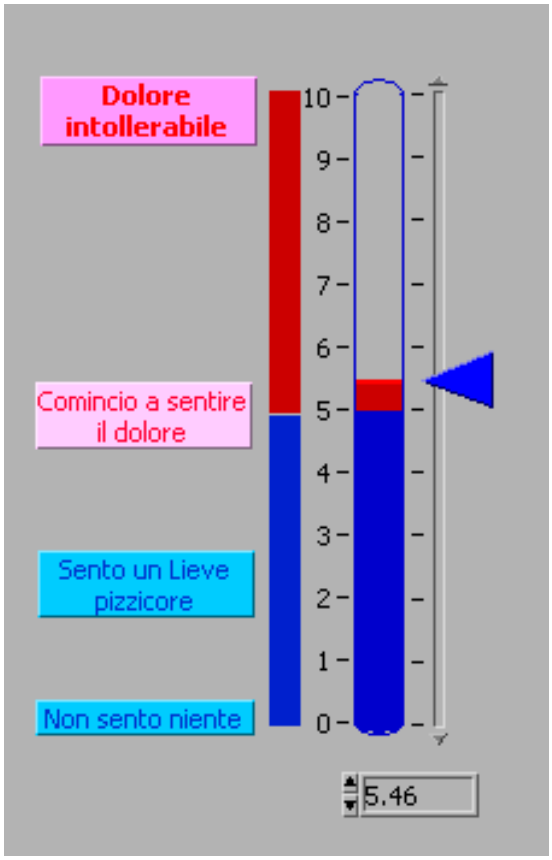
Datum: _____

Die Schmerzschwellebestimmung – Intensität

| | **Serie1-Ansteigen** | **Serie1-Absteigen** | **Serie2-Ansteigen** | **Serie2 -Absteigen** |
|---|---|---|---|---|
| **8 mA** | | | | |
| **7,5 mA** | | | | |
| **7 mA** | | | | |
| **6,5 mA** | | | | |
| **6 mA** | | | | |
| **5,5 mA** | | | | |
| **5 mA** | | | | |
| **4,5 mA** | | | | |
| **4,0 mA** | | | | |
| **3,5 mA** | | | | |
| **3 mA** | | | | |
| **2,5 mA** | | | | |
| **2 mA** | | | | |
| **1,5 mA** | | | | |
| **1 mA** | | | | |
| **0,5 mA** | | | | |
| **0 mA** | | | | |

Mittelwert der Intensität: _____

### *8.4.2. Sheet for the Pain Threshold for the Study 3*



## 8.5. Curriculum Vitae

### PERSÖNLICHE INFORMATIONEN


| | |
|---|---|
| Name | Marta Andreatta |
| Adresse | Sterenstraße 10, D-97074, Würzburg |
| Telefone | +49/0176 64158534 |
| E-Mail | marta.andreatta@mail.uni-wuerzburg.de |
| Staatangehörigkeit | Italienisch |
| Geburtstag | 16. Januar 1979 |


### ARBEITSERFAHRUNG

- 01.03.2009 – 31.08.2009 Wissenschaftliche Hilfskraft bei der WIVW GmbH – Würzburger Institut für Verkehrswissenschaften
- 1.9.2006 – 30.11.2006 Wissenschaftliche Hilfskraft an der Universität Würzburg
- 01.09.2005 – 25.09.2005 Grundschullehrerin
- 05.2004 Wahlhelferin Europawahl.
- 01.07.1997 – 21.07.1997 Betreuerin von 20 Kindern, 7-8 Jahre). Beim Caritas von Trento (Italien).

### AUSBILDUNG

- 20.09.2009 Workshop “Advanced fMRI Signal Processing Using Statistical Parametric Mapping (SPM)”
- 08.01.2009 Teilnahme am Programm des projektbezogenen Personenaustauschs
- 19.10.2008 VIGONI-Programm . Italienisch und Deutscher Austausch
- 10.04.2008 -13.04. 2008 Workshop “Biopsychology of Emotion“
  Workshop “Comparative Research on Emotion Processing”
- 27.03.2008 – 30.03.2008 Workshop “Emotions”
- Seit 01.2008 Vertreterin der Studenten des Graduiertenkollegs Emotions.
- 6.10.2007 -9.10.2007 Workshop “Methods of Affective Neuroscience”
- 27.04.2007 – 29.04.2007 Workshop “Brain and Behavior Days”

| | |
|---|---|
| • 12.03.2007 -17.03.2007 | Spring School „fMRI in der psychologischen Forschung am BION“ |
| • Seit 01.2007 | Doktorandin des Graduiertenkollegs Emotions am Lehrstuhl für Psychologie I der Universität Würzburg, |
| • 01.09.2005 - 01.03.2006 | Klinisches Praktikum im Dienst für Drogenabhängige (Servizio per le Tossicodipendenze - Ser.T.), Rovereto (Italien). |
| • 01.03.2005 – 01.09.2005 | Forschungspraktikum am Lehrstuhl für Psychologie I der Universität Würzburg |
| • 01.09.2003 –01.12. 2004 | Abschluss des Diplomstudiengangs Psychologie, Diplomarbeitsthema “Cortical reorganisation of language in aphasic patientes: a study with ERP” |
| • 09.1998 – 01.12.2004 | Psychologiestudium an der Universität Padova (Italien) |
| • 01.08.2002 – 01.06.2003 | Erasmusprogramm an der Norges Teknisk-naturvitenskapelige Universitet (NTNU) in Trondheim (Norwegen) |
| • 01.10.2002 – 15.04.2002 | Klinisches Praktikum am psychiatrischen Krankhaus „Ai Colli“ in Padova (Italien) |
| • 06.1998 | Liceo Psicosociopedagogico, Instituto Magistrale F. Filzi, Rovereto (Italien) |

**PREISE**

| | |
|---|---|
| • 11.03.2009 | Posterpreis 2009 der Fachgruppe „Biologische Psychologie und Neuropsychologie“ der Deutschen Gesellschaft für Psychologie |

**ZUSATZQUALIFIKATIONEN**

| | | | |
|---|---|---|---|
| MUTTERSPRACHE | Italienisch | | |
| Weitere Sprache | ENGLISCH | DEUTSCH | NORWEGISCH |
| Lesefähigkeiten | GUT | GUT | |
| Schriftliche Fähigkeiten | SEHR GUT | SEHR GUT | |
| Mündliche Fähigkeiten | GUT | SEHR GUT | GRUNDLAGEN |

| | |
|---|---|
| **EHRENAMTLICHE TÄTIGKEITEN** | Mitglieder des AK Internationales in Würzburg (Deutschland). |

## 8.6. Publications

### *8.6.1. Papers*

Kenntner-Mabiala, R., **Andreatta, M.**, Wieser, M. J., Mühlberger, A., & Pauli, P. (2008). Attention and affect modulate pain perception independently as indicated by somatosensory evoked potentials. *Biological Psychology 78,* 114-122.

### *8.6.2. Conference Proceedings*

**Andreatta, M.**, Mühlberger, Gerdes, A. B. M., Wieser, M. J., Gerber, B., Pauli, P. (2009). The rewarding aspect of an aversive event. *Society for Psychophysiological Research (SPR),* Berlin, Germany.

**Andreatta, M.**, Mühlberger, Gerdes, A .B. M., Wieser, M. J., Gerber, B., Pauli, P. (2009). Event-timing könnte die Angstreaktionen modulieren. *Arbeitstagung Psychophysiologie und Methodik (APM),* Leipzig, Germany.

**Andreatta, M.**, Mühlberger, Gross, C., Weyers, P., Pauli, P. (2009). Could the context become a predictor of an aversive stimulus? *Cognitive Neuroscience (CNS)*, San Francisco, US.

**Andreatta, M.**, Mühlberger, A., Gerber, B., Pauli, P. (2008). Aversive unconditioned stimuli can inhibit defensive system. *Human Brain Mapping (HBM),* Melbourne, Australia.

**Andreatta, M.**, Mühlberger, A., Gerber, B., Pauli, P. (2008). Can a painful stimulus induce either conditioned avoidance or conditioned approach? *Internation Congress of Psychology (ICP),* Berlin, Germany.

**Andreatta, M.**, Mühlberger, A., Gerber, B., Pauli, P. (2008). Event timing may turn punishment to reward: a biopsychological study in humans. *FENS,* Geneve, Switzerland.

**Andreatta, M.**, Mühlberger, A., Gerber, B., Pauli, P. (2008). Kann durch Timing Strafe zu Belohnung werden? Eine biopsychologische Studie. *Psychologie und Gehirn,* Magdeburg, Germany.

**Andreatta, M.**, Mühlberger, A., Pauli, P. (2008). Aversive unconditioned stimuli can inhibit the defensive system. *International Symposium on Learning, Memory and Cognitive Function. Mechanisms, Pathology and Therapeutics.* Valencia, Spain.

**Andreatta, M.**, Kenntner-Mabiala, R. & Weyers, P. (2007). Pain modulates valence ratings of affective faces: a pilot study. *Psychologie und Gehirn,* Dortmund.

**Andreatta, M.**, Kenntner-Mabiala, R., Mühlberger, A. & Pauli, P. (2007). Puó uno stimolo avversivo condizionare sia il comportamento di evitamento che quello di avvicinamento negli esseri umani? *Congresso Annuale Associazione Italiana di Psicologia,* Como.