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The Roles of the Amygdala and the Hippocampus
in Fear Conditioning

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Abstract

The amygdala, a small structure located deep bilaterally in the medial temporal lobe, is the key structure for the emotional processing and storage of memories associated with emotional events, especially fear. The structure has also been shown to enable humans and animals to detect and respond to environmental threats. Fear conditioning became the main model to examine the neural substrates of emotional learning in mammals and specifically in rats'. With the fear conditioning method, researchers can tests rats', responses to aversive stimuli during the delivery of a cue and then measure how the responses change after learning of the association between the stimuli and the cue. After learning of the two stimuli, the delivery of a cue alone will prompt a fear response in the rats. The fear response can also be elicited by placing the rats in the same chamber in which the aversive stimuli has previously been experienced, which depends on both the amygdala and the hippocampus. Where the amygdala stores the memories of stimulus related to fear, the hippocampus seems to hold all the fear memories in relation to contextual information about the stimulus. The aim of this paper will be to make a comprehensive overview of internal neural processes of both the amygdala and hippocampus and the interaction between the two structures during fear conditioning, to see how the structures separately work to overlap emotion and memory processes.

Keywords: context, fear, memory, conditioning, amygdala, hippocampus

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Introduction

One major goal of cognitive neuroscience is to identify and gather more understanding of the neural substrates that underlie learning and memory. In order to survive in an environment, an organism must be able to learn the structure of the environment, such as the existence of stimuli and its relationship to other stimuli. It is because of the organisms' ability to memorize and learn about stimuli that the organism possesses the ability to adapt its behavior to the surrounding environment. To be able to study the learning processes of an organism, scientific methods have been used in which the organism is exposed to two different kind of stimuli during a period of time. First is an organism exposed to a learning experience, then at a later time, is the organism re-exposed to a specific experience that reveals the modification that the learning experience has produced. The question is whether the relation between the two experiences has modified the organism in a manner that can be detected at the second time of experience. The study of this learning process is mostly known as *classical conditioning*, a process in which an organism's innate response to a stimulus becomes expressed in response to a previously neutral stimulus (Rescorla, 1988).

Classical Conditioning

The pioneering discovery of classical conditioning was accidently discovered by the Russian physiologist Ivan Pavlov when he studied dogs salivating in response to being fed. What he noticed was that his dogs would begin to salivate when the lab assistant entered the room even when not bringing them food. According to the contemporary terminology, the food worked as an *unconditioned stimulus* (US) whereas the salivary secretions worked as an *unconditioned response* (UR). These findings contributed to the understanding that there are some innate

species-typical responses that will be expressed automatically, as the ability to learn and

memorize according to the environment (McLeod, 2007).

Pavlov understood that the dogs somehow had learned to associate the food with his lab

assistant and that the change in behavior must be the result of learning processes. Thus, the lab

assistant that originally had been a neutral stimulus (didn't evoke any response) had become

associated with a US, the food. He conducted an experiment in which he instead of the lab

assistant, rang a bell as a neutral stimulus when delivering the food for the dogs. The experiment

created an association between the neutral stimulus (the bell) and the US (food), and the neutral

stimulus became a *conditioned stimulus* (CS) as the response was learned. Pavlov and his

systematic studies of classical conditioning are now one of the most widely used methods to

study learning and memory processes (McLeod, 2007).

In behaviorist terms, classical conditioning suggests that learning is an association that is

created between two stimuli presented close in time (McLeod, 2007; Diamond & Rose, 1994).

Specifically, an initially neutral stimulus (CS), such as a sound, is paired with a motivationally

stronger stimulus (US), either a reward or a punishment (food or shock), that in turn evokes a

behavioral response. As a result of the learning of relationship between the CS and US, the

organism shows an enhanced response to the CS, also referred to as a *conditioned response* (CR),

and the outcome is that an association has been created between the CS and US (Diamond &

Rose, 1994; Rescorla, 1988). The evocation of the CR is a good indicator that learning processes

have occurred (Bouton & Moody, 2004).

Fear Conditioning

Fear conditioning is a form of classical conditioning, the difference, is that the US is unpleasant and used to investigate the neural mechanisms of emotional learning across a wide range of species, especially in rats. The fear conditioning experiment tests a rat's response to a specific stimulus and measures how the responses change after learning. In the pre-training stage, the rat receives a light or a tone (CS), which is a neutral stimulus that does not evoke any fearful response in the rat. However, as the rat receives a foot shock (US), it elicits a startle- or freezing response (Davis, 1992; Phelps, 2006; Phelps & LeDoux, 2005). The startle and freezing response serves as a defensive behavioral system critical for protection against dangerous environmental threats, thus interrupting the ongoing behavior and increases attention toward the aversive stimuli. The rat does not have to learn to perform these responses, thus the response is implicit and an innate warning of danger (Bolles & Fanselow, 1980; Davis, 1992; Fendt & Fanselow, 1999). These natural fear responses to the shock are the UR. After a few pairings of the CS and the US, the rat learns to associate the two, a process also known as *acquisition* (Blanchard & Blanchard, 1972). Eventually, the CS alone, without the US will prompt a fear CR in the rats. The CS and the resulting CR can subsequently be unpaired by presenting the CS alone many times, without the US. The neutral stimulus will then stop being associated with the aversive stimulus and just be a neutral stimulus again. At this point, the CR is considered *extinguished* (Davis, 1992; Fendt & Fanselow, 1999; Phelps, 2006; Phelps & LeDoux, 2005).

By the use of fear conditioning, researchers have made a lot of progress in mapping the neural circuitry and processes critical for fear learning. Converging evidence now indicates that the amygdala is the central structure in the circuitry for the expression of fear CRs (Davis, 1992; Phelps, 2006). This research has identified not only the amygdala's critical role in fear

conditioning, but also the involvement of specific synaptic connections in the acquisition and

storage of memories of fear conditioning in animals (Davis, 1992; LeDoux, 2000, 2012;

McGaugh & Roozendaal, 2002; Rodrigues, Schafe, & LeDoux, 2004; Fanselow & Poulos, 2005)

and humans (LeDoux, 2012; Phelps, 2006; Phelps & LeDoux, 2005).

In animal studies, the convergence between CS and US occurs in the lateral nucleus of the

amygdala, which leads to the formation of the CS-US association. Another important region that

has been shown to be a site of fear acquisition and memory storage is the central nucleus of the

amygdala (LeDoux, 2012; Wilensky, Schafe, Kristensen, & LeDoux, 2006). Studies of fear

conditioning on human subjects are consistent with the findings of animal models. Thus,

functional magnetic resonance imaging (fMRI) has provided evidence of the occurrence of an

increased blood oxygenation level-dependent (BOLD) signal in the amygdala when neutral

stimuli became associated with an aversive event. The CR, which has been measured using *skin

conductance responses* (SCRs) as an indication of arousal to the CS event, correlated with the

magnitude of amygdala activation (Büchel, Morris, Dolan, & Friston, 1998; LaBar & Phelps,

1998; Phelps, 2006). All of these results provide evidence of the amygdala's involvement in fear

conditioning in humans.

Context- Dependent Fear Conditioning

An emotional CR can also be elicited by placing an animal in the same chamber in which

the aversive US has previously been experienced, but without the occurrence of the US. Thus, the

memory for the CS has become context-specific, or as it will be referred to here, *contextual fear

conditioned* (for reviews see: Fanselow, 2000; Phillips, & LeDoux, 1992; Squire, 1992). In this

situation, the CR is not elicited by a stimulus that is explicitly paired with the US, it's instead

paired with the contextual information of the chamber where the US occurred. The UR are then

re-experienced when the animal return to the chamber (Hobin, Goosens, & Maren, 2003; Phillips, & LeDoux, 1992).

Although most research has examined the processes of the amygdala, considerable evidence has been obtained which indicates that the hippocampus is crucial for the development of emotionally significant fear conditioning in relation to contextual information (Phelps, 2004; Bouton & Moody, 2004; Hobin et al., 2003; Phillips, & LeDoux, 1992). The amygdala has been shown to interact with the declarative (conscious) memories of the hippocampus to strengthen the unconscious and conscious memories for emotional events (Phelps, 2004). For example, a woman is walking down the street, she sees a neighbor's dog further down the road that she is afraid of and with this knowledge in mind, she decides to change her path. The reason she's afraid of this particular dog could be for the reason the dog once bit her and, in that case, her fear response was acquired through normal fear conditioning. The dog acted as a CS and became paired with the dog bite (US), resulting in fear (UR) and an acquired fear response to avoid the dog (CR). However, it is also possible that the woman has learned about the emotional significance of stimuli through a neighbor that previously has told her that the dog is dangerous and might bite her. In this case, she has learned the emotional significance of the aversive stimuli unconsciously through verbal communication that requires the hippocampus for acquisition and retrieval when the fearful stimulus is present. This type of verbal learning, in which humans learn to fear and avoid a stimulus through instruction of an aversive experience, shows the amygdala-mediated strengthening of memory for specific events, which depends on the hippocampus (Phelps, 2004). For this reason, the hippocampus has been the target of several studies investigating the contextual acquisition and extinction of fear conditioning but also the retrieval of the fearful memories. As the hippocampus receives inputs from all the cortical areas that

integrate sensory information, this might underlie the function of contextual processing (Bouton,

Westbrook, Corcoran, & Maren, 2006; Phillips, & LeDoux, 1992).

Aim of the Paper

The amygdala and hippocampus, two medial temporal lobe structures, are two independent

systems, which both seem to have unique functions in fear memory formation. The hippocampus

is necessary for the acquisition of declarative knowledge of the emotional significance of a

stimulus whereas the amygdala's crucial role concerns the acquisition and expression of a fear

CRs (Phelps, 2004). Evidence that these two memory systems are independent comes mainly

from animal studies and case studies of patients with lesions of the amygdala or hippocampus,

which have provided evidence of a double dissociation between the structures (Bechara et al.,

1995; Phelps, 2004). Studies have been conducted to examine how the hippocampus influence

the amygdala but also to examine how the amygdala influence the hippocampal-dependent

memory for emotional stimuli (Bass, Nizam, Partain, Wang, & Manns, 2013). It has been

suggested that the amygdala modulates the consolidation of contextual memories through the

release of stress hormones that follows from the stimuli (McGaugh & Roozendaal, 2002). An

emotional reaction to an event releases stress hormones that in turn elicit emotional responses

appropriate for the context or survival and is, therefore, more likely to be remembered for a

longer time (McGaugh & Roozendaal, 2002; Phelps, 2004).

Anyone who has returned to the neighborhood where they grew up can feel the striking

experience of long-lost memories coming back in vivid detail to their mind. Events that are

personal and encountered during a specific context are better recalled when the person is located

in the same context again (Smith & Bulkin, 2014). The same goes for emotional arousing fearful

experiences, these experiences are rapidly learned and can also be stored and remembered for a

long period of time. The context in which stimuli are presented is essential for learning and

memory processes and findings indicate that the hippocampus is crucial for the acquisition of

contextual information (Smith & Bulkin, 2014). The environmental contexts work as cues that

allows subjects to retrieve the information associated with the experiences without the

interference from experiences learned in other contexts. These associations that are created can

be so strong that simply asking the subjects to think about the environment can recall the

emotional experience (Smith & Bulkin, 2014). The fear-conditioning paradigm has become an

excellent model for experimental studies trying to unravel the processes and mechanisms

underlying context-informational memories. Contextual fear conditioning could also be defined

as the display of anxiety in a context that has previously elicited anxiety to the subjects (Baas,

Nugent, Lissek, Pine, & Grillon, 2004). Scientists have suggested that the amygdala and the

hippocampus both play a significant role in anxiety disorders. An investigation of how fearful

memories are stored and retrieved during contextual fear conditioning by the amygdala and

hippocampus might provide a better understanding of various anxiety disorders and phobias

(Baas et al., 2004).

Most of the research have examined the roles of the amygdala and hippocampus separately

in studies of fear conditioning. Research on the interaction of the two structures have not yet been

conducted to the point that it's a well-established field of research which means that the

interactions between the amygdala and hippocampus still possess some unanswered questions.

This final thesis will be conducted as a comprehensive overview of the two memory systems'

functional neural substrates separately, to see how the amygdala and the hippocampus contribute

to contextual fear memories in their specific way. The thesis will also examine the interaction

between the two structures, as a way of explaining how emotion overlap memory processes. The

structure of the paper will be divided into three main sections that will investigate pre-existing

neuroscientific studies conducted in the field of fear conditioning and its dependency on

information about environmental context. The first section will examine the neural substrates of

the amygdala and its relation to negatively emotionally arousing stimuli and fear conditioning.

The paper will first look at the history of the amygdala research and its relation to fear and

threatening stimuli (for reviews see: Davis & Whalen, 2001; LeDoux, 2012). The second section

will then go over to examine the neural substrate of the hippocampus, and in which way

informational knowledge of the environmental contexts contribute to fear conditioning. Whereas

the amygdala stores the memories of stimulus related to fear, the hippocampus seems to hold the

contextual stimulus about fear. The functions of the structures indicate that the amygdala and

hippocampus possess complementary roles in fear conditioning. Several theories have been

considered over the years on what the hippocampal function might be in fear conditioning. In the

hippocampal section, some of these will be examined and discussed to see what the function of

the neural substrates of the hippocampus might be (Fanselow, 1980; Keleman & Fenton, 2010;

Maren, Aharonov, & Fanselow, 1997; Young, Bohenek, & Fanselow, 1994). The last section will

discuss studies of the interaction between the amygdala and hippocampus, especially the

interaction between the dorsal hippocampus and the basolateral complex of the amygdala (Bass et

al., 2013). The main knowledge of the CS-US association observed in contextual fear

conditioning have been provided by research on rodents and patients with damage to the

hippocampus and amygdala structure, so studies of this sort will have the main focus in this

overview. In the end, a conclusive discussion will take place, to summarize, what the overview

has found in relation to the neural substrates of the amygdala, hippocampus and the structures

involvement during fear conditioning processes in relation to contextual cues.

Amygdala's Role in Fear

The amygdala is a small almond-shaped mass of gray matter located bilaterally deep in the medial temporal lobe adjacent to the anterior portion of the hippocampus. The structure has been suggested to be crucial for emotional processing in both humans and animals and was first described in relation to studies of Klüver-Bucy syndrome (Klüver & Bucy, 1937). This syndrome is characterized by a set of unusual behaviors observed in monkeys after bilateral removal of the temporal lobes. Monkeys with such lesions developed psychic blindness (an inability to understand the significance of visual stimuli), and approached, ate and orally examined dangerous objects, such as snakes, without hesitation. The monkeys also acquired a change in social and emotional behavior, such as loss of fear and increased tameness toward the caretakers. Thus, the monkeys that once avoided the presence of their caretakers started to approach and make contact with them after the bilateral removal of the temporal lobes (Klüver, & Bucy, 1937; for review see: Davis & Whalen, 2001; LeDoux, 2012). At the time, very few systematic analyses had been made of the changes in behavior. However, approximately 20 years later, Weiskrantz (1956) attempted to localize the effects that contribute to the behavioral changes in the monkeys. Weiskrantz's (1956) study assessed the role of the amygdala as the structure for the avoidance behaviors seen in the monkeys during fear conditioning and he established that learning and memory motivated by fear requires an intact amygdala. Thus, Weiskrantz (1956) found that amygdalectomy impaired the identification of fearful stimuli and performance of fear avoidance (for review see: LeDoux, 2012).

After the pioneering discovery of the amygdala's role in identifying fearful stimuli and fear avoidance behavior, several studies were conducted to investigate further why amygdala lesions altered reactions to threatening stimuli. Researchers, such as Blanchard and Blanchard (1972),

were able to demonstrate that bilateral damage to the rat amygdala played a crucial role in the

changes of reaction toward threatening stimuli, which in their experiment was a cat. Thus,

amygdala lesions eliminated the freezing response toward the cat and made the rats approach the

cat without fear (Blanchard & Blanchard, 1972). They also obtained results that lesions to other

limbic structures, such as the hippocampus, led to other responses in the rats with reduced

freezing and altered avoidance behavior. Blanchard and Blanchard (1972) study in rats showed

the occurrence of what would later be known as the acquisition of contextual fear conditioning.

Although most of the research on fear conditioning has been conducted on rodents, some

researchers have conducted neuropsychological studies of the fear-conditioning paradigm on

humans. One neuropsychological study conducted by LaBar, Gatenby, Gore, LeDoux and Phelps

(1998) used fMRI in parallel with the classic fear conditioning method to examine the activity in

the human amygdala during the phases of acquisition and extinction in fear conditioning. Healthy

participants were randomized into two groups, one experimental and one control group. The

experimental group was presented with one visual stimulus (CS) that was paired with an

electrical shock (US) to the wrist, whereas the subjects in the control group was presented with

the CS only (LaBar et al., 1998). FMRI scans on the participants were acquired during both the

acquisition phase and extinction phase during the fear conditioning task. The results of the

experimental group showed a greater activity in the amygdala for both the phases during the task.

In addition, the results demonstrated an average of greater activity in the right hemispheric

amygdala of the subjects participating in the study during both the acquisition and extinction

phases. The results of this study provided further evidence for amygdala function across species

during associative emotional learning tasks, but also some differences in hemispheric activation

during the task (LaBar et al., 1998).

The role of the amygdala has also been examined in human case studies in which patients with damage to the amygdala have shown an impaired ability to recognize emotions in facial expression, especially in expressions related to fear (Adolphs, Tranel, Damasio, & Damasio, 1995; Adolphs et al., 1999). One study by Adolphs et al. (1995) investigated the consequences of bilateral and unilateral lesions to either right or left amygdala and to what extent the lesions were associated with facial fear expression. The subjects of the study were divided into four groups: one female participant with bilateral amygdala damage (patient SM046), one group of subjects with unilateral left amygdala damage, one group of subjects with unilateral right amygdala damage and a control group with healthy individuals (Adolphs et al., 1995). The participants were instructed to recognize facial expressions presented on a screen that included faces of happiness, sadness, surprise, disgust, anger and fear and neutral faces. The subjects were also instructed to rate the emotional intensity of the expressions. The results of the study demonstrated that SM046 had greater impairments in judging the intensity of faces showing fear compared to the other groups in the study (Adolphs et al., 1995). These results suggest that bilateral amygdala damage and not unilateral amygdala damage impairs the judgment of the intensity and recognition of fear expressions, but that the recognition of non-fearful emotions remains intact in these patients. In addition to the facial expression task, SM046 was asked to draw facial expressions from memory. SM046 was able to draw all requested facial expressions, except fear, because she could not bring to mind what a fearful expression looked like, which can be explained by impairments in visual recognition related to the emotional meaning of fear (Adolphs et al., 1995). The study of Adolphs et al. (1995) revealed a great deal of knowledge about the bilateral amygdala and its importance in fear detection, recognition and the retrieval of fear related knowledge.

Another study conducted by Adolphs et al. (1999) examined nine subjects with bilateral damage to the amygdala on identical tasks with identical stimuli, which provided a detailed and quantitative evaluation of their ability to recognize emotions in facial expressions. Studying a relatively large number of rare individuals with bilateral damage and comparing them with healthy control groups, gave a unique opportunity to examine why damage to this area impaired the recognition of fear in facial expressions and explored the reason these individuals became impaired on the specific task. Compared to the control groups, the subjects with bilateral amygdala damage were significantly impaired in recognizing facial expressions of fear. Thus, the subjects had low rating scores for the recognition of negative emotions, but their ratings on happy faces appeared to be normal (Adolphs et al., 1999). Adolphs et al. (1999) analysis of the rating scores pointed towards that subjects with bilateral damage to the amygdala were impaired because they did not understand the signaled emotion during certain facial expressions, because it appeared that the subjects did not misread the emotions, but rather were uncertain of their evaluation. Adolphs et al. (1999) suggested that the results of the study demonstrated that the underlying mechanism of the impairment in subjects with bilateral damage was an inability to trigger knowledge retrieval of the specific emotion, when presented with emotional expressions related to negativity. Overall, the results were consistent with the hypothesis that the amygdala has a crucial role in the rapid triggering of knowledge related to threats and danger, including in the facial expressions of humans.

A study by Anderson and Phelps (2001) was conducted to examine whether the human amygdala also influenced the likelihood that certain stimuli reached awareness and modulate perception. The colleagues used a method based on the paradigm called the *attentional blink effect* that is observed during a rapid and serial visual presentation (RSVP) of significant stimuli,

or in this case words. Subjects were asked to attend selectively to a target stimulus presented in

the stream of the RSVP of words. After identifying the target word, there is an impairment in

awareness for a subsequently presented second target, this impairment in attention toward the

second stimuli is the effect of the attentional blink (Anderson & Phelps, 2001). Anderson and

Phelps (2001) used the attentional blink method to investigate the importance of the amygdala in

modulating perception in a patient (S.P.) with bilateral damage to the amygdala and in 10 patients

with unilateral lesions of the right or left amygdala (five patients with damage to the right

hemisphere and five patients with damage to the left hemisphere). S.P. is a female that had her

right amygdala medically removed as a result of anteromedial temporal lobe resection because of

epilepsy. The resection included partial removal of the anterior, middle and inferior temporal gyri

and a complete removal of the hippocampus, parahippocampus and projection fibers to their

posterior extent. However an additional lesion was also observed in the left amygdala region,

which indicates that the amygdala damage had been bilateral (Anderson & Phelps, 2001).

    In the experiment of Anderson and Phelps (2001), the subjects were asked to identify and

report green target words among a stream of black words during the RSVP of words. The

recorded data demonstrated that both S.P. and the other subjects had a normal attentional blink

effect. In addition, the subjects with unilateral lesions identified the negative words in the stream

with greater accuracy than neutral words. However, unlike these subjects, S.P. demonstrated

almost no identification of the negative words compared to the neutral words (S.P. 25% versus

controls 72.6%) (Anderson & Phelps, 2001). The findings of the study supported the idea that the

influences of the human amygdala extend to perceptual encoding, which in turn influences the

extent to which the emotionally significant stimuli reach awareness and shapes the perceptual

experience. Thus, if the second target in the RSVP was an emotionally arousing stimulus,

subjects were less likely to miss it. Furthermore, the occurrence of the visually presented

negative words during the RSVP evoked greater activity in the amygdala compared to the

presented neutral words (Anderson & Phelps, 2001).

Anatomy of the Amygdala

Although the amygdala is a small structure, it possesses complex internal structures

composed of several distinct groups of cells: the lateral (LA), basal and accessory basal nuclei,

collectively termed the basolateral amygdala (BLA), and they form the primary sensory interface

of the amygdala. The basolateral amygdala is in turn surrounded by several structures including

the central (CE), medial and cortical nuclei that collectively make up the amygdaloid complex.

The two main structures, the basolateral amygdala, and the amygdaloid complex have together

come to be called the amygdala (Figure 1; Maren, 2001; Pitkänen, Savander, & LeDoux, 1997).

The complex structure of the amygdala contributes to the function of receiving input from all

sensory modalities and send output to various regions in the brain related to emotional learning.

This section will have the main focus on only a few of these subregions, namely the most

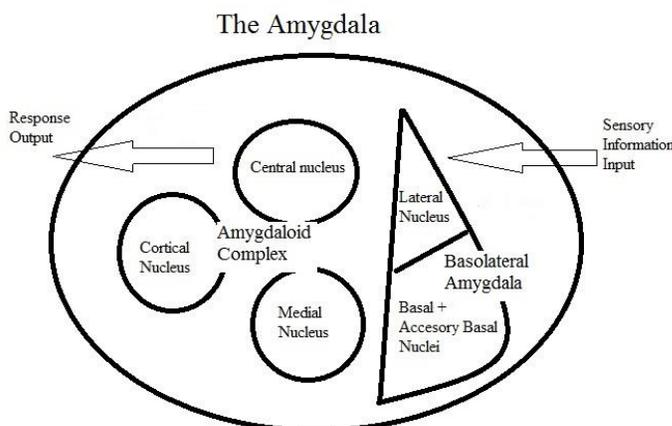relevant to fear conditioning (Davis & Whalen, 2001).
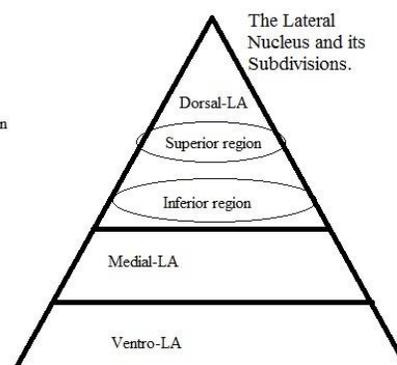

Figure. 1. The amygdala and its subdivisions.   Figure. 2. The LA and its subdivisions.

The BLA receives sensory information from the thalamus, hippocampus and cortex, which contribute to that the structure possess the function to transmit signals to activate the appropriate brain regions that are relevant to the sensory information signal (Davis & Whalen, 2001). The LA, within the BLA, is an important structure for emotional learning that can be divided into three major subdivisions: the dorsal-, ventro- and medial- LA (LeDoux, 2007). The dorsal-LA can be even further sub-divided into a superior and inferior region: where cells in the superior part have been shown to be involved in the fear learning process and the inferior part is involved in the long-term storage of fear memories (Figure 2; LeDoux, 2007). The LA is, in general, referred to as the gatekeeper of the whole amygdala. Thus, the structure receives inputs from all the different sensory systems such as the auditory, visual, somatosensory (including pain), olfactory and taste system (LeDoux, 2007).

During emotional learning, sensory inputs from the neutral and US converge in the LA (Wilensky et al., 2006). For example, in reward conditioning, the tone or light (CS), paired with the food (US), creates a positive change in neural transmission in the LA, which in turn sends a signal to the striatum that encodes the association. The positive change in neural transmission leads to approach behavior. If, however, the neutral CS is paired with an aversive shock (US), as in fear conditioning, the change in neural transmission becomes negative and transmits a signal to the CE, which serves as the output nucleus of the amygdala. The CE produce an increase in attention toward the stimulus, as well as producing fear, which is important for the expression of fear CR (Davis & Whalen, 2001; Wilensky et al., 2006). Expression of these fear responses involves connections from the CE to the brainstem, which controls specific behaviors and physiological responses, for instance freezing when confronted with danger (LeDoux, 2007). The connections between CE and other areas of the brain makes it a major output gateway in which

the inputs received through the LA, can leave and travel to the other brain regions. Thus, the

connection between the CE and the striatum are involved in the avoidance of aversive stimuli, for

example, escape from danger (LeDoux, 2007).

A study conducted by Wilensky et al. (2006) demonstrated that the CE, like the LA, plays

an important role in the acquisition and the consolidation of a fear memory. The fact that neurons

in the CE and LA both show increased responsiveness to stimuli after that they have been paired

with a US indicates that both the structures are involved in the convergence of the CS-US

association. This activity has in particular been measured in dorsal LA neurons. Thus, the

processes of the convergence in this subarea responds to both the US and CS (Fendt & Fanselow,

1999). However, even though the CE receives both CS and US information, the CE participation

in fear learning remains uncertain and debatable. Thus, it is possible that the encoding occurs in

parallel with the CS-US association processes in the LA (Wilensky et al., 2006).

That large lesions of the amygdala impair fear conditioning in animals has long been

known (see: Blanchard & Blanchard, 1972). Nader, Majidishad, Amorapanth, & LeDoux (2001)

conducted a study which used a method that produced electrolytic lesions in several of the major

nuclear regions in the amygdala of the rat, especially those that connect the CS sensory

processing and control the expression of the CR, to examine which amygdala nuclei that were

necessary and crucial for the acquisition of fear CR (freezing and startle responses) to auditory

stimuli. They used electrolytic lesions because of the need for a restriction to small regions of the

bilateral amygdala with minimal infringement on neighboring nuclei (Nader et al., 2001). The

rats that received lesions to the LA, CE or the entire amygdala were dramatically impaired on the

fear conditioning trials in which the auditory CS was paired with the aversive US. However, only

lesions to the LA blocked the acquisition of responses altogether, which suggest that the LA is

crucial for mediating the fear learning. Lesions to the CE, blocked fear conditioning in general, because of its role as the motor output in which learned information travel through to be able to access the circuits controlling freezing or startle responses (Nader et al., 2001). Electrolytic lesions to other areas in the amygdala failed to interfere with fear conditioning to auditory CS, which suggests that LA and CE, with the connections between them, are sufficient on their own to mediate fear conditioning and for the acquisition of fear conditioning responses (Nader et al., 2001).

The internal connections between the CE and LA allow the structures involved in receiving the inputs and generating the outputs to communicate, and this circuitry in turn contributes to the activation of the *hypothalamic-pituitary-adrenal* (HPA) *axis* which results in stress reactions (LeDoux, 1994). Fear conditioning has been demonstrated to activate the HPA axis, which subsequently sends a signal to the pituitary gland that in turn releases the *Adrenocorticotropic hormone* (ACTH). The signal is also sent to the adrenal gland, which releases steroids that help the animal or human to cope with the stressful event (LeDoux, 1994). In this sense, the connection between the LA and the CE is required and crucial for the expression of learned fear behavior and an animal's survival. Thus, when the LA is damaged, the ability to acquire and express previously learned fear becomes impaired and damage to the CE, impairs the ability to respond to fear during fear conditioning (Schafe, Nader, Blair, & LeDoux, 2001).

Amygdala Involvement in Memory Storage

All types of learning involve changes in the synaptic strength between neurons in the brain's circuitry, these changes can either be strengthened or weakened over time depending on their activity (Bliss & Collingridge, 1993). *Long-term potentiation* (LTP) has been the main experimental model for examining the synaptic basis for learning and memory in both the

amygdala and hippocampus, and is induced by the activation of the *N-methyl-D-aspartate*

(NMDA) receptor complex. LTP is an enduring increase in synaptic transmission that is induced

by high-frequency stimulation. To trigger the induction of LTP, two inputs, one neutral and one

strong, need to occur nearly simultaneously. LTP as a research method has provided a

mechanism for the investigation of the association formation between stimuli and research has

found considerable evidence that LTP can be found in all of the major sensory input pathways to

the amygdala (Bliss & Collingridge, 1993; Schafe et al., 2001).

Over the years, experimental studies and research on rodents have mapped the inputs and

outputs of the amygdala's nuclei. The LA and CE have been proposed to be the nuclei most

important in mediating fear conditioning. Plenty has also been learned about the neural

mechanisms that underlie the synaptic plasticity in the LA during the convergence of the CS-US

association in fear learning. When the CS is re-experienced, it activates the same potentiated

synapses in the output gateway of CE as the US, which control the fear CRs (LeDoux, 2007;

Schafe et al., 2001). The success of defining the structures and connections of fear conditioning,

in parallel with LTP studies of synaptic plasticity, have combined provided new knowledge and

insights into the mechanisms underlying acquisition and consolidation of fear memories (Schafe

et al., 2001). During fear conditioning, the neuronal activity in the sensory inputs to the amygdala

is enhanced in a similar way to what is seen in the artificial induction of LTP in the structure,

which suggests that an LTP process occur in the LA which could be the underlying factor for fear

conditioning to occur (Schafe et al., 2001).

Evidence of parallels between LTP and fear conditioning has been found during in vitro

studies that have made a detailed analysis of the neural and cellular mechanisms of amygdala

plasticity. LTP can be induced in a weak synaptic pathway if the activity is paired with activity in

a stronger pathway. This associative LTP is a mechanism for the formation of the CS-US

association in the amygdala during fear conditioning (Maren, 1999). The neutral stimulus (CS)

isn't aversive in a manner that initiates the pathways in the amygdala, but the strong aversive

stimulus (US) can produce a fear state in the mammalian brain. The result of fear conditioning is

that the weak CS pathway, is paired with the stronger US pathway and that the neurons then are

potentiated by the associative LTP, which becomes reactivated later on because of the occurrence

of the same CS. The available evidence in this field supports a role for LTP in the amygdala

during fear conditioning, but further research is required to determine if the amygdala's circuits

exhibit the synaptic LTP while learning (Maren, 1999).

There is no doubt that studies on the effects of lesions in the amygdala in animals and

humans is consistent with the idea of the amygdala's involvement in mediating affective

influenced memory. Furthermore, it have also been demonstrated that the hormones

glucocorticoid and *epinephrine* (an adrenal hormone related to *norepinephrine*), influence

memory storage and consolidation of the amygdala (Maren, 1999; McGaugh, Cahill, &

Roozendaal, 1996; McGaugh & Roozendaal, 2002). It has been suggested that experiences which

arouse negative emotions are more rapidly acquired and stored for a much longer time compared

to less arousing experiences because of the crucial information of dangerous environmental

threats for survival that the experiences convey (McGaugh et al., 1996; McGaugh & Roozendaal,

2002). Evidence on this comes from experiments on mammals that demonstrate the release of

adrenal stress hormones, which has a crucial role in consolidating lasting memories. Both the

glucocorticoid and epinephrine effects in memory modulation involve activation of

norepinephrine within the amygdala. However, the two hormones influences the amygdala

differently. Thus, the glucocorticoids are able to enter the brain through the blood-brain barrier

and activate adrenal steroid receptors, whereas epinephrine does not freely pass through the blood-brain barrier and appears to only indirectly mediate the effects on memory consolidation by activating *B-adrenergic receptors* (McGaugh et al., 1996). In addition, have studies also shown that there a high density of glucocorticoid receptors located in the hippocampus (McGaugh et al., 1996; McGaugh & Roozendaal, 2002).

In sum, the historically important studies emphasize two main aspects of the amygdala, (1) its role in social behavior, as seen in Klüver-Bucy syndrome and (2) its role in emotional learning and memory, as seen in Pavlovian fear conditioning (Blanchard & Blanchard, 1972; Klüver & Bucy, 1937; LaBar et al., 1998). The amygdala has been demonstrated to also possess a major role in the triggering of knowledge related to threats and danger, including facial expressions in humans related to fear (Adolphs et al., 1995, 1999). The memory function of the amygdala emerges from the complex internal structures composed of the distinct groups of cells. Especially the BLA, as the primary sensory interface of the amygdala and the LA, within the BLA, as the nucleus that receives all the different sensory stimuli. As well as the CE that serves as the output nucleus of the amygdala. The LA and CE of the amygdala have been proposed to be the nuclei most crucial in mediating fear conditioning (LeDoux, 2007; Maren, 2001; Pitkänen et al., 1997). In addition, it has also been demonstrated that fear conditioning induces an increase in the synaptic transmission in the amygdala during learning and that the release of adrenal stress hormones influences the memory storage and consolidation (LeDoux, 2007; Maren, 1999; McGaugh et al., 1996; McGaugh & Roozendaal, 2002; Schafe et al., 2001)

The Role of the Hippocampus in Fear Conditioning

The hippocampus, located in the medial temporal lobe, is a primary memory system in humans, necessary for declarative or episodic memories, which is those memories that can be

brought to mind at will. As the amygdala modulates the acquisition and the storage of fear

memories, the hippocampus forms declarative representations of events with emotional

significance (Phelps, 2004). Emotion overlap memory as these two independent memory systems

interact. This phenomenon can mainly be seen by the use of fear conditioning in human subjects

with lesions to either the amygdala or the hippocampus. In a classic fear conditioning method in

human subjects, a neutral blue square becomes paired with an aversive shock to the wrist of the

patients. A patient with amygdala damage will fail to show a physiological fear response (SCR)

toward the blue square, even though they can report that the blue square predicted the shock.

However, if the same method is used on a patient with hippocampal damage the results will be

the opposite, the subjects will demonstrate a physiological arousal response (SCR) to the blue

square, but are not able to consciously recollect that the blue square had previously been paired

with the aversive shock to the wrist (Phelps, 2004). These results demonstrate a double

dissociation of conditioning and declarative knowledge relative to the human amygdala and

hippocampus. Double dissociation means that damage in brain structure X impairs function A

but spares function B and that damage to brain structure Y, instead impairs function B but not

function A. The occurrence of a dissociation can then lead researchers to make more accurate

inferences and hypotheses of brain structures function and their spatial localization, in this case,

the amygdala and hippocampus (Bechara et al., 1995; Phelps, 2004).

 Bechara et al. (1995) used a method similar to the classic fear conditioning method

mentioned above when examining three subjects with distinct bilateral brain lesions: SM046 with

damage to the amygdala but intact hippocampus, WC1606 who had damage to the hippocampus

but intact amygdala, and patient RH1951, who had damage to both of the structures. In addition

to the three experimental subjects, four normal participants were used as comparable controls.

Bechara et al. (1995) used two conditioning experiments in the study, a visual-auditory conditioning experiment and an auditory-auditory conditioning experiment. These experimental conditioning tasks were carried out to measure SCRs during the training phase, the conditioning phase and during two extinction phases. SM046's ability to acquire conditioned SCRs to the CS was completely blocked by the bilateral damage to the amygdala, however her ability to provide accurate factual information regarding the stimuli (CS) by the US remained intact in both of the experiments. WC1606 exhibited the opposite results; he generated normal SCRs to the CS in both of the experiments, but he could not provide any factual information regarding the CS-US pairing (Bechara et al., 1995). These results are consistent with the results obtained by the method mentioned in the section above (see Phelps, 2004). However, participant RH1951 with bilateral hippocampal and amygdala damage was entirely blocked in his ability to acquire conditioned SCRs or factual knowledge (Bechara et al., 1995). The obtained results from the study all pointed toward two independent memory systems; the amygdala which is essential for the emotional association and response whereas the hippocampus has been suggested to be involved during the CS-US learning of both the factual information regarding the stimuli and the relations among the contextual cues.

At this point, the thesis will address the role of the hippocampus in Pavlovian fear conditioning in relation to memory formation of contextual information of the environment. Studies examining the amygdala-hippocampal interactions have mainly focused on how the amygdala influences hippocampal-dependent episodic memory, for emotional stimuli. As already mentioned, it has been demonstrated that the amygdala influences the attention and alters the encoding of emotional events as they receive priority in memory formation, and that it is the main site of plasticity for the formation and storage of fear memories (see: Anderson & Phelps, 2001).

But the hippocampus is also important for certain forms of memory formation and has been

studied in both humans and rodents using fear conditioning methods, which has demonstrated a

more complex and selective role for the hippocampus during fear conditioning (Sanders, Wiltgen,

& Fanselow, 2003).

The knowledge about which structures and connections that are important for memory

formation mainly come from case studies of neurological patients with memory impairments and

experimental research of animals, especially rodents and monkeys (for review see: Squire, 1992).

The amnesic patient R.B. was the first documented human subject who had memory impairment

as a result of lesions limited to the hippocampus because of an ischemic event (loss of blood

supply to the brain) during open-heart surgery. After his death, a closer examination of R.B.'s

brain revealed damage located in the bilateral CA1 region of the hippocampus (Squire, 1992).

Lesions limited to the hippocampal area in rodents and monkeys has been documented to produce

memory impairments in the mammals on a variety of tasks, such as declarative, spatial memory

tasks but also relational memory tasks that require learning of the relationship between different

stimuli (Squire, 1992). These results have all together provided compelling evidence that the

mammalian hippocampus has an essential role in memory formation.

Behavioral data has suggested a unique role for the hippocampus in contextual fear

conditioning and the model has become a major research paradigm for testing hippocampal-

dependent learning in rodents (Fanselow, 2000). Context is referred to as the set of circumstances

or facts that surround a specific event or situation. In a contextual fear conditioning experiment,

the rat is re-placed in the chamber where it previously experienced an aversive shock (US). But,

in this case, the CS that causes the freezing or startle behavior (CR), are the contextual cues that

compose the chamber. The rat is typically given a few minutes to explore the contextual

information of the chamber prior to the delivery of the aversive shock, this time period is

essential for the rat to acquire a robust context conditioning. If the period to explore the

environment is too short the rat will not be conditioned to the specific context, which is known as

immediate shock deficit (Fanselow, 2000). The freezing response (CR) of the rats is a result of the

learned relation between the context stimuli and the stimuli of the aversive shock (US), as a way

to try reduce the impact of the shock. The resulting response is a reliable variable to measure

during observation of the phenomena (Fanselow, 2000). The freezing response that follows a

shock could be a CR to the contextual cues given at the time of the shock, in that case should it

be CS or context specific. However another possibility has been suggested that the freezing

response is a delayed UR. If that would be the case, then a delay between the aversive shock and

time of testing should reduce the freezing response (Fanselow, 1980).

Fanselow's (1980) study rejected the idea of a delayed UR by a method based on two

variables: context dependency and time dependency. Following a delay of 24 hour the UR should

be lost, but CRs should still be present. The rats were placed in a chamber where they received

mild (0.5 shock intensity) or moderate (1 shock intensity) intensity shocks, a group of control rats

were also used that received no shock (0 shock intensity) in the chamber. To be able to test the

context dependency variable, the rats were either replaced into the same chamber as they

previously had experienced the aversive shock or in an alternative (no shock) chamber. To test

the time dependency variable, the rats were replaced into the chambers, either immediately after

the aversive shock or 24 hours later (Fanselow, 1980). All the rats that had been presented the

aversive shock froze, and as the intensity of shock increased so did the freezing response in the

rats. Furthermore, both the intensity (0.5 and 1) testing groups of rats' demonstrated reduced

freezing behavior in a context different from the chamber they were presented the shock

(Fanselow, 1980). The results of the context dependency test demonstrated that the freezing

response is a CR to the contextual cues present at the time of the shock. The time dependency test

demonstrated similar results: the shocked rats froze more when re-placed into the chamber after

the 24-hour delay, than the rats re-placed and tested immediately after the first shock (Fanselow,

1980).

It is important to understand that fear memories do not depend on a single anatomical

structure but arise from interactions among different structures and their neural circuitry. The

amygdala and hippocampus are only a few of the structures involved in the neural circuitry of

fear memories, and their inputs and output systems allow mammals to adapt and respond to the

environment and its threats. The place of hippocampus has been suggested to be a sensory and

motor relay but also a site of information storage, which in turn makes the region a key structure

that processes the experience with environmental events and produces the adaptive behaviors in

response to those events (Sanders et al., 2003).

Hippocampus as an Input and Output Structure in Fear Conditioning

Studies with place cells within the hippocampus of the rodents have demonstrated that the

region receives multimodal sensory information that process visuospatial information, but also

olfactory and auditory stimuli (Sanders et al., 2003). The question is whether the hippocampus

only relays the obtained sensory information to any other regions in the neural circuitry of fear

conditioning? Studies have been conducted, as a way of examine whether pre and post-training

lesions of the hippocampus disrupt contextual fear conditioning in rats as a way to investigate the

question (Kim & Fanselow, 1992; Maren et al., 1997; Young et al., 1994). The obtained results

from the studies have provided supportive evidence to the idea of a sensory role for the

hippocampus in contextual fear conditioning. The major findings demonstrated that pre-training

lesions indeed blocked the transmission of sensory information during acquisition and that post-

training lesions impaired the recall of the conditioned stimuli. However, the studies also obtained

results that argued against a sensory role for the hippocampus (Kim & Fanselow, 1992; Maren et

al., 1997). Rats that received lesions delayed for a period of time after the training phase retained

their contextual fear memories, these results demonstrated that the hippocampus might have a

time-limited role in forming the contextual association of fear memories and that the role of

hippocampus could have another function (Kim & Fanselow, 1992).

Another hypothesis that has been suggested is that the hippocampus could be a relay for

creating the defensive responses seen in studies of contextual fear conditioning. Thus, studies

have shown that lesions and other manipulations of the hippocampus create changes of motor

behavior in fear conditioning and other related tasks (for review see; Sanders et al., 2003).

Studies as such have demonstrated that hippocampal lesions in addition to impairments in context

fear conditioning also resulted in an increased locomotor activity (Young et al., 1994). This

increased activity could point out the importance of hippocampus during the organization of fear

responses, however, neurochemical manipulation studies has demonstrated that the role of the

hippocampus might not be as simple as expected. A hippocampal study conducted by Bailey,

Tetzlaff, Cook, He and Helmstetter (2002) reported that manipulation of the *gamma-*

aminobutyric acid (GABA) transmission caused fear response prior to the presentation of the

aversive foot shock. The study demonstrated that the GABA-agonist RY024 caused both reduced

context conditioning and an increased fear behavior in the animals. The manipulation of the drug

concluded that the processes of the hippocampus could not be behavioral output processes and

that the hippocampus' role is not a motor output structure in fear conditioning (Bailey et al.,

2002). Could the hippocampus role instead be to store critical information that is important for fear conditioning?

Hippocampus as a Memory Structure in Fear Conditioning

The hippocampus has been suggested to be critical for learning the environmental factors that are important for fear conditioning. It has also been suggested that the contextual CS in relation to fear, is the combination of the many stimuli (visuospatial, olfactory and auditory etc.) in the environment that need to be memorized of an organism for a higher chance of survival. The various stimuli in the environment must be associated and combined with each other to create the context. It is here that the role of the hippocampus has also been proposed to behold the function to create contextual representations, which then can be associated with the US, or in the case of fear conditioning, the aversive shock (Sanders et al., 2003). Lesion to the hippocampus produces both retrograde and anterograde amnesia of contextual fear conditioning. However as it has already been demonstrated, lesions of the hippocampus after a period of time does not create any impairment in contextual fear conditioning (Sanders et al., 2003; Young et al., 1994). The temporally limited retrograde amnesia of contextual conditioning suggests that the memory of the conditioning after a period of time becomes independent of the hippocampus and at some point after conditioning the representation of the context becomes permanently stored elsewhere, probably the neocortex (Young et al., 1994). The role of the hippocampus may be to assist the progress of association formation between the different contextual factors and not the location of the memory processes necessary for the learning and performance of contextual fear (Young et al., 1994).

A suggestion made of the process by which the information transfer to the permanent storage region has been that the mechanism responsible for the transfer is LTP. It has been

demonstrated that NMDA-antagonist's block LTP in contextual fear conditioning (Young et al., 1994). Young et al. (1994) found that infusion of NMDA in the hippocampus one week before conditioning produced anterograde amnesia in the rats but also that blockage of NMDA receptors during training also provided impairments in conditioning.

Maren et al., (1997) made an extensive investigation of the effects of NMDA lesions to the dorsal hippocampus during acquisition phase and expression phase in fear conditioning. Electrolytic lesions of the dorsal hippocampus has been shown to produce impairments during both the acquisition and expression phase of contextual fear conditioning. The study of Maren et al., (1997) investigated whether damage to the neurons in the dorsal hippocampus could be responsible for these deficits? Maren et al., (1997) made lesions to the dorsal hippocampus prior to training in one group of rats and after the training phase in another group of rats. The lesions were made either one week before or at several different times after the fear conditioning training phase. Post-training lesions produced temporally graded retrograde impairments in contextual fear that indicated that the hippocampus has a time-limited role (Maren, 2008; Maren et al., 1997). That is, post-training lesions one day after conditioning resulted in greater deficits in context freezing whereas post-training lesions made 100 days after produced mild deficits. However, unlike Young et al.,'s (1994) study, the lesions prior to training did not affect context fear and both pre- and post-training lesions of the dorsal hippocampus produced an impairment in tone-conditioning (Maren et al., 1997).

There are many controversies about the hippocampus and its role in storing contextual memories (Sanders et al., 2003). Its role seems more related to processes such as time-limited retrieval, and mnemonic processes that underlie the acquisition and consolidation of contextual fear and which likely involves the NMDA receptors. This line of evidence fits well with the data

provided from studies of humans with damage to the hippocampus: that the structure has an

essential role in memory formation, and provide much more evidence that the hippocampus, in

fact, plays a crucial role in contextual learning (Sanders et al., 2003).

Hippocampal ensembles represent the contexts

New findings of the nature of hippocampus and context presentations indicate that an

ensemble of hippocampal neurons encode each context that is encountered. New analysis

techniques have made the characteristics and functions of the hippocampal ensembles more

tractable and easier to record (Smith & Bulkin, 2014). Keleman and Fenton (2010) found in their

study that the hippocampus could hold two distinct contextual representations and then alternate

between them when necessary. The researcher of the study used a two-frame place avoidance

task, in which the rats were placed on a continuously rotating arena and trained to organize their

behavior to two concurrently relevant contexts: one stationary and one rotating. The researchers

implanted electrodes into the hippocampus and by doing this, they were able to record how

information about locations in these two contexts is organized in the action potential discharge of

the ensembles of hippocampal cells (Keleman & Fenton, 2010). The findings of the study

demonstrated that the hippocampus switched between the different ensembles and that each

representation was most active when the rat was close to the relevant danger zone. The

hippocampus treated the environments as two distinct behavioral contexts, whereas one triggered

avoid behavior, and then switched between these two representations when needed (Keleman &

Fenton, 2010). This function of the hippocampus provides a mechanism for retrieving the

appropriate avoidance response. Thus, the ensemble carries essential information about the

context and becomes engaged as a coherent unit (Smith & Bulkin, 2014). Studies have overall

supported the idea that an ensemble of hippocampal neurons represents any new context a subject

encounters. The hippocampus can then quickly switch between the stabilized ensembles to distinguish among contexts (Smith & Bulkin, 2014).

Even if it has been established that hippocampal ensembles correlate with the context and the appropriate behavior, it has been difficult to establish a direct causal link between the neural firing and memory formation. Advances to Pavlovian fear conditioning methods have contributed to overcoming these difficulties better, thus they use a molecular neuroscience technique that allows them to directly manipulate the hippocampal ensembles with a drug or a light. This has led to techniques that can determine if reactivation of an ensemble causes the retrieval of a specific contextual memory (Smith & Bulkin, 2014).

A study that was able to demonstrate the causal link between the activity in the hippocampal ensembles and fear-conditioned memories were Ramirez et al.,'s (2013) study. Ramirez et al., (2013) study used artificial means of optogenetic (light) to stimulate specific hippocampal neural ensembles located in dentate gyrus (DG). During the first day, mice were exposed to one kind of context (A) and the active neurons were tagged with *channelrhodopsin*, a protein that serves as a sensory photoreceptor sensitive to optical stimulation. The day after, the mice were given an aversive foot shock in another context (B) while the labeled neurons from context A became optically stimulated and reactivated (Ramirez et al., 2013). The effects of the artificial reactivation of the fear learning from context A in the novel context B resulted in that the mice expressed a freezing response even though they had never been shocked in the novel contextual environment. The results of Ramirez et al. (2013) study demonstrated that hippocampal DG neurons can serve as CS during fear conditioning when the neurons are reactivated by artificial means during the delivery of a US. The consequence of the reactivation is a formation of a false fear association between the stimuli that was not presented at the time of

the US delivery. The reactivation of the hippocampal ensemble located in DG was a successful

substitute for the physical context and demonstrated that the ensembles doubtlessly represented

the context (Ramirez et al., 2013). The study of Ramirez et al., (2013) was the first study in the

field to be able to directly manipulate the neural memory representation of fear conditioning and

indicate that conditioned fear can be retrieved without the context in which the neural firings

naturally occurred in. How the ensembles are formed to the new context in the first place is not as

well understood at this point (Smith & Bulkin, 2014).

Smith and Bulkin (2014) have suggested that the acquisition of contextual information is

important for preventing a mnemonic interference that belong to unrelated contexts. The context

works as a retrieval cue and as returning to a familiar context, the environmental cues results in

the priming of relevant memories and making it easier to retrieve the right responses for the

environment. Smith and Bulkin (2014) presented a working model as an attempt to explain the

coding processes that occur: A unique hippocampal ensemble is recruited to represent a new

context that a subject has come across. With experience, the context becomes coded and

associated with the specific stimuli, behaviors and events that occur in that context and the

hippocampal ensemble that activates becomes associated with the representation of the memory

and the memory-specific responses. As the subject later return to the specific context the

hippocampal ensemble associated with the context automatically reactivates and primes the

retrieval of the relevant memory and responses, by doing this irrelevant memories and responses

do not interfere (Smith & Bulkin, 2014).

As discussed earlier, previous accounts demonstrated theories where the hippocampus

connected the various neocortical elements that composed the memories and then brought them

together into distributed contextual and episodic memories. Subsequently, reactivation of the

hippocampus leads to automatic retrieval of the coded memory. Smith and Bulkin (2014) model

also hold the idea that reactivation of hippocampal ensembles automatically reactivates the

memory representations. However, unlike the previous ideas Smith and Bulkin (2014) suggest

that the role is not to link the individual components of the episodic memory, but rather to link

the memories to the right context. These ideas are a better explanation for the results obtained

from studies of hippocampal lesions in animals and human subjects, which is the inability to

associate the memories with the right context during hippocampal damage (Smith & Bulkin,

2014).

The Interaction between the Amygdala and the Hippocampus

The paper has already made it clear that for contextual fear conditioning to proceed, an

animal must form a coherent representation of a context, a representation of emotional experience

and an association between the two stimuli need to be formed. The circuits mediating contextual

fear conditioning have been extensively described in this thesis, but the precise contribution to

what makes up for the phenomena has not been fully elucidated. To help clarify and better

understand the functions of the hippocampus and the amygdala in contextual fear conditioning, a

cellular compartment analysis of temporal activity using fluorescence in situ hybridization

(catFISH) has been used to visualize the neuronal populations involved in the process

(Zelikowsky, Hersman, Chawla, Barnes, & Fanselow, 2014). CatFISH has demonstrated to be an

exceptional tool for investigating the behavior of neuronal ensembles in multiple brain structures

involved in contextual fear.

The dorsal hippocampus has already been demonstrated to be an important region for

contextual fear (see: Maren et al., 1997). However, the role of the dorsal hippocampus during

acquisition of the aversive component of contextual fear conditioning and the interaction between the amygdala and hippocampus is yet to be settled.

Zelikowsky et al. (2014) conducted a study that combined the method of catFISH with the contextual fear conditioning model to investigate the neuronal ensembles in the amygdala, the hippocampus, especially the dorsal hippocampus, and the prefrontal cortex (PFC). The PFC's role in the acquisition of contextual information was examined in the study in relation to the context-shock association. The study specifically examined whether the representation of an environment would alter different brain regions when the animal formed a context-shock association (Zelikowsky et al., 2014).The rats in the study were either delayed fear-conditioned for five minutes (experimental group) or immediately shocked (control group). Twenty minutes after the fear conditioned tests, the rats were re-exposed to the same context to test for fear memory recall. Subsequent catFISH analyses revealed that neuronal ensembles in the BLA only became reactivated if the animals had been fear conditioned previously. In addition, it was revealed that place cells located in the dorsal hippocampus became reactivated during both context tests. Zelikowsky et al. (2014) suggested that the structure encoded the spatial information presented in the environment. Thus, the activation of place cells is usually thought to require movement through a specific location, but the findings of the study suggest that these cells undergo activity even without any movement that allow recognition of the previously experienced environment. Furthermore, it was revealed that PFC also possessed a supporting role in both fear and contextual processes (Zelikowsky et al., 2014). The study found that the same population of cells located in the BLA that were active during Pavlovian fear conditioning, became reactivated during retrieval, these findings are consistent with the idea that these cells are involved in fear expressions. The findings also provided compelling evidence that the spatial and

emotional properties of an environment are encoded by the dorsal hippocampus and BLA respectively (Zelikowsky et al., 2014). The data, taken together, reveal a dissociable contribution that the amygdala and hippocampus, with PFC included, are involved in the integration of both the contextual acquisition and emotional processing. The necessity of the structures interactions to produce appropriate and adaptive behavior appeared with clarity.

Research with rodents and human subjects has indicated that the improvement of memory during emotional arousal events depends on the BLA. A study conducted by Bass, Partain and Manns (2012) was able to demonstrate that direct electrical stimulation of the BLA increased acquisition of fear memories for specific events and objects. However, the BLA activation is still very unclear at this point, but as already mentioned, BLA possesses broad connections throughout the brain that receives sensory information from several brain structures (Davis & Whalen, 2001).

One primary structure known to receive direct projections from the BLA is the hippocampus (Bass et al., 2013; Zelikowsky et al., 2014). The structure has been shown to be important for the acquisition of declarative memory but also demonstrated synaptic plasticity following activation of the BLA (Bass et al., 2013; Phelps, 2004). A study that investigated whether BLA-mediated enhancement of memory for specific events and objects depended on the hippocampus was conducted by Bass et al. (2013).The rats in the study received infusions of either muscimol or saline into the hippocampus prior to an object recognition memory task. Some of the rats also received brief electrical stimulation to the BLA immediately encountered the objects. The rats in the saline condition group remembered objects well but not when the encountered object had not been directly followed by BLA stimulation when tested one day after the experiment. (Bass et al., 2013). However, no memory enhancement was observed in the

muscimol condition group of rats. The results of Bass et al.,'s (2013) study demonstrated that brief electrical stimulation to the BLA when the rats encountered environmental objects, resulted in those object being better remembered than encountered objects without BLA stimulation. The obtained results suggested that the activation of the BLA can prioritize memories for events and objects by enhancing memories that the organism can benefit from. Memory functions as such depend on both direct and indirect connections between the amygdala and the hippocampus and the interaction between the two memory structures (Bass et al., 2013).

Discussion

This paper was conducted to make a comprehensive overview of the amygdala and the hippocampus roles in fear conditioning by investigating the internal neural processes of both the amygdala and hippocampus separately. The paper also discussed the interaction between the amygdala and the hippocampus and how emotion and memory processes overlap. Taken together, fear conditioning has become an important research paradigm for investigating the neural substrates of memory and learning processes in both humans and animal subjects. The model has the equipment for studying the fear condition of several subjects simultaneously during the learning of the CS-US association and the contextual information provided at the time of learning. Because of the success of the observations and recorded data on the phenomenon of fear conditioning, there has been an increased understanding of the amygdala and hippocampus and their importance for the acquisition, storage and retrieval of fear memories in relation to environmental contexts (for review see: Maren, 2008).

The amygdala has long been proposed as the major region in the brain that contributes to the perception of fear in both human and animals, hence the structure is specialized to predict dangerous stimuli and to trigger the appropriate physiological responses to dangerous and

threatening stimuli. Klüver and Bucy (1937) were the first to document the consequences of bilateral damage to the temporal lobes in monkeys. It was not until 20 years later, Weiskrantz (1956) pinpointed that it was the amygdala specifically that contributed to the emotional changes seen during fear conditioning. From that point, studies of the amygdala escalated in numbers.

In humans, studies on patients with amygdala damage provided further knowledge on the role of the amygdala in emotional and fear learning (Adolphs et al., 1995, 1999). Studies of this kind have documented that patients are impaired in their ability to store the relevant emotional information in their long-term memory, which in turn affects the subjects' behavioral fear responses (such as SCRs) and their concept of fear. The importance of rapidly and efficiently evaluating motivationally significant stimuli, especially dangerous stimuli, is crucial for the survival of both animals and humans and depends on maintaining of specialized neural structures that provide the easier detection of dangerous stimuli. Healthy humans had a benefit for the perception of aversive stimuli compared with stimuli of neutral content in which a patient with bilateral amygdala damage hasn't (Adolphs et al., 1995, 1999; Anderson & Phelps, 2001). However, these patients' abilities to provide factual information about what stimuli (CS) predicted the aversive shock (US) remains intact. The obtained results from the case studies confirm the crucial role of the amygdala in the storage of emotionally arousing information and of generating SCRs to aversive stimuli. Thus, the occurrence of SCRs has been shown to correlate with the participants' CS-evoked activity in the amygdala.

There is also supportive evidence that patients with hippocampal damage are impaired in the opposite manner, in their inability to provide factual information about the learning of the CS-US association and the prediction of the aversive stimuli (US). While nevertheless maintaining a concept and response to fear in general, in the sense that they can produce SCRs to aversive

stimuli and acquire conditioned fear in general, but cannot verbalize the stimuli that are associated with the aversive stimuli (Bechara et al., 1995; Squire, 1992). Because of these studies, there is supporting evidence that healthy subjects normally acquire knowledge of the US-CS association and contextual knowledge during fear conditioning.

Although case studies of human patients with these specific lesions are rare, the use of animal research models has provided further discoveries of the role of hippocampus and amygdala functions during fear conditioning. Animal research has generated a great deal of information regarding the neurobiology of fear memories. Thus, the success of the fear conditioning in studying the memory processes and expression of conditioned emotional responses to stimuli, together with the fact that avoidance behavior toward threatening stimuli was a function of the amygdala, provided progress in mapping the circuitry of the brain regions where the CS and aversive US converge and form the fear conditioning in animals (Davis, 1992; LeDoux, 2000, 2012; Rodrigues et al., 2004; Fanselow & Poulos, 2005; Weiskrantz, 1956) and humans (LeDoux, 2012; Phelps, 2006; Phelps & LeDoux, 2005).

Amygdala activity is at its strongest during the acquisition phase when the emotional association is formed, because of the high activity in the most crucial nuclei of the amygdala during fear conditioning: the CE and LA (Phillips & LeDoux, 1992). The LA, the sub-nucleus of the BLA, and the CE has been demonstrated to be key structures during acquisition and retrieval of conditioned fear responses (Wilensky et al., 2006). Lesions to the CE and LA nuclei block the acquisition of fear to both cues and contexts. Thus, lesion to the rats' LA impairs the ability to acquire and express learned fear and lesions to the rats CE impairs the ability to respond to the fearful stimuli (Phillips & LeDoux, 1992; Schafe et al., 2001). The obtained results are conclusive: the amygdala is important for mammals in order to predict and avoid dangerous

situations and react appropriately for survival in their everyday life. However, neuroscientific research in rodents has over the years documented some critical differences and similarities between the hippocampus and amygdala and their processes during acquisition, consolidation and retrieval of fear memories.

The experimental model of LTP has provided extensive knowledge of the synaptic basis for fear and learning processes. Maren et al.'s (1997) study of NMDA lesions to the hippocampus demonstrated that retrograde amnesia of contextual fear had a time-limit role. Thus, rats that received 100 days delayed post lesions after the training phase retained their retrieval of contextual fear memories of the environmental cues. These results suggest that the hippocampal structure processes are related to time-limited retrieval and that the memories were stored elsewhere, probably in neocortex (Maren et al., 1997; for review see: Maren, 2008). Furthermore, LTP during fear conditioning has been documented to not only occur in the hippocampal routes, but also in the amygdala routes of information processing (Maren et al., 1997; Schafe et al., 2001; Young et al., 1994). Hippocampal studies have reported enhanced activity in the brain region during recall of memories. The evidence demonstrates that the value of contexts affect the strength of the fear memory acquisition of the amygdala. For example, the subject is presented to dangerous environmental stimuli, and the amygdala then creates a direct attention toward the dangerous stimuli (Phillips & LeDoux, 1992; Schafe et al., 2001; Wilensky et al., 2006).

The newest findings to the field about the nature of hippocampus and its relation to fear conditioning is that each encountered context is coded by a specific ensemble of hippocampal neurons and that the structure can switch between the ensembles to distinguish between the different contexts. This process provides a mechanism for retrieving the appropriate avoidance behavior during contextual fear conditioning when re-exposed to relevant environmental stimuli

(Keleman & Fenton, 2010; Smith & Bulkin, 2014). It's here that, Ramirez et al.'s (2013) study provided further knowledge to the field of fear conditioning in relation to contextual information. Their laboratory design made it possible to study the formation and retrieval of both genuine and false contextual memories during fear conditioning by the use of artificial means of optogenetics. This new technique can investigate fear conditioning circuits in a way that cognitive studies that use behavioral and functional magnetic resonance techniques cannot register, even though the technique is highly restricted to laboratory settings (Ramirez et al., 2013). The fact that reactivation of cells that were active during the formation of the fear memory, induced the retrieval of the memory that first became associated with the emotional arousing event (the US) could form a false memory, could be pioneering for the treatment of anxiety disorders and phobias.

The development of new and more innovative techniques for research will help to bridge the gap between animal and human research. Thus, fear is a powerful emotion, and to be able to study its responses in human subjects, the researcher must be careful to not crossing what is considered ethically right to study in human subjects (Maren, 2008). The techniques used in animal studies, cannot always be used on human subjects. For example, studies of rodents has used many behavioral measures as an index of fear CRs, including freezing responses, startle responses and exploratory behavior as locomotion when the rodents are introduced to novel contexts (Maren, 2008). However, the most used index is the freezing response, which can be observed at the delivery of the CS, when the aversive CS-US association has been learned. In human subjects, the main index of fear CRs have been the occurrence of SCRs at the delivery of CS, as a prediction of the following aversive shock (US).

An attempt to generate clinical measures from the research in rodents, which use context as a fear conditioning cue, to human subjects' and anxiety patients could be the use of virtual reality techniques. A virtual reality could function as a tool to create different contextual environments while keeping the patients in laboratory settings, where the patients can be studied in controlled surroundings and further extend the understanding of fear conditioning processes in relation to contextual information in human subjects (Baas et al., 2004; Smith & Bulkin, 2014).

Despite the gap between animal and human contextual fear conditioning studies, the fear conditioning paradigm will continue to be an incredibly useful model for the understanding of the function of amygdala and hippocampus neural substrates that underlie the acquisition, consolidation and retrieval of emotional memories (Maren, 2008). However, with the use of new and improved techniques researchers will not only know that manipulation of a specific neural circuit will have the predicted effect on a behavioral response but also better understand how the interaction between the amygdala and the hippocampus contribute to the memory processes (Bass et al., 2013; Maren, 2008; Smith & Bulkin, 2014). More studies of the interaction between the amygdala and the hippocampus is crucial for a better understanding of the phenomena. To conclude: even though further investigation of the interaction between the amygdala and hippocampus still is needed to understand fear conditioning to the fullest, the fear conditioning paradigm has been an incredibly useful model to understand the neural substrates of the phenomena (Bass et al., 2013). As researcher gathers more data and a broader understanding of the phenomena of fear conditioning, the possibility to generalize the obtained knowledge to the population will be increased. Better clinical treatments for phobias and anxiety disorders, such as posttraumatic stress disorders, might in the future be acquired by a broader knowledge of how contextual fear memories affect people.

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