Amygdala Involvement in Aversive Conditioning

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"If you would be a real seeker after truth, it is necessary that at least once in your life you doubt, as far as possible, all things."

- Rene Descartes (1596-1650)

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Abstract

Research over the past several decades has revealed that the amygdala is involved in aversive, or fear, conditioning. However, the precise nature of this involvement remains a matter of debate. One hypothesis suggests that disrupting amygdala function eliminates the storage of memories formed during aversive conditioning, eliminating the production of internal responses that alter the expression of observable behaviors. Alternatively, lesions or inactivation of the amygdala may impair the modulation of memories in other brain regions and disrupt the ability to perform certain observable behaviors.

The experiments reported in the present thesis examined these arguments by making multiple behavioral measures during exposure to unconditioned (US) or conditioned (CS) aversive cues. Amygdala activity was inferred from changes in c-Fos protein expression or activity was temporarily suppressed with muscimol injections. The relationship between the behavioral measures and the role of the amygdala in producing them was examined.

Amygdala neurons expressing the c-Fos protein tracked exposure to the US and CS but did not coincide with expression of freezing. Temporary inactivation of the amygdala with muscimol injections before presentation of the US or exposure to the CS attenuated the expression of freezing and active place avoidance; two incompatible behaviors. Finally, temporary inactivation of amygdala activity blocked freezing, place avoidance, and memory modulation produced by the same posttraining exposure to an aversive CS.

Since amygdala activation alone was not sufficient to produce freezing and inactivation of the amygdala eliminated freezing, place avoidance, and memory modulation, the results cannot be interpreted as reflecting a direct role for the amygdala in production of observable behaviors. The results also preclude the idea that memory modulation is the only function of the amygdala. Rather, the results of all three studies suggest that the amygdala stores an aversive representation of the US which promotes the expression of various behaviors, possibly through the production of internal responses reflecting an aversive affective state.

Resumé

La recherche des dernières décennies a révelé que l'amygdale est impliquée dans le conditionnement aversif, ou de la "peur". Cependant la nature précise de cet engagement reste une question de débat. Une hypothèse suggère que le fait d'interrompre cette fonction de l'amygdale élimine l'emmagasinage de mémoires formées pendant le conditionnement aversif, éliminant ainsi la production de réponses internes qui altèrent l'expression de comportements observables. Une hypothèse alternative suggère que les lesions ou l'inactivation de l'amygdale peuvent endommager la modulation de mémoires dans d'autres régions du cerveau et interrompre la capacité d'exécuter certains comportements observables.

Les expériences rapportées dans la thèse actuelle ont examiné ces arguments, en prenant de multiples mesures comportementales pendant l'exposition aux stimuli inconditionnés ("US") ou conditionnés ("CS") aversifs. L'activité de l'amygdale a été conclue par les changements dans l'expression de protéine c-Fos ou l'activité a été temporairement éliminé avec des injections de muscimol. Le rapport entre les mesures comportementales et le rôle de l'amygdale dans la production de ces mesures a été examiné.

Les neurons de l'amygdale exprimant la protéine c-Fos ont été activés par l'exposition aux US et CS mais leur activation n'a pas coïncidé avec l'absence de mouvement chez le rat. L'inactivation temporaire de l'amygdale avec les injections de muscimol a contré l'effet de la présentation des US ou CS sur l'action d'éviter l'endroit ainsi que sur l'augmentation d'absence de mouvements; deux comportements incompatibles. Finalement, l'inactivation temporaire d'activité de l'amygdale a bloqué l'action d'eviter l'endroit, l'augmentation d'absence de mouvement, et la modulation de mémoires produites par la même exposition post-entraînement à un CS aversif.

Puisque l'activation de l'amygdale n'était pas suffisante pour produire l'augmentation d'absence de mouvement, et puisque l'inactivation de l'amygdale a éliminé l'augmentation d'absence de mouvement, l'action d'éviter l'endroit, et la modulation de mémoires, les résultats ne peuvent pas être interprétés comme reflétant un rôle direct pour l'amygdale dans la production de comportements observables. Les résultats excluent aussi l'idée que la modulation de mémoire est la seule fonction de l'amygdale. Les résultats des trois études

suggèrent plutôt que l'amygdale emmagasine une représentation aversive des US qui promeut l'expression de divers comportements, probablement via la production de réponses internes reflétant un état affectif aversif.

Preface

Although it is well accepted that the amygdala is involved in aversive conditioning, its exact role is controversial. The present thesis consists of three manuscripts reporting experiments that examine this controversy and contribute to its resolution.

One group of researchers suggests that the amygdala encodes and stores memories that underlie the behavioral changes associated with aversive conditioning. According to this idea, information about associations between environmental cues and aversive events stored in the amygdala leads to an aversive affective state ("fear"). The affective state promotes behaviors normally associated with fear-producing cues. Lesions or inactivation of the amygdala disrupt this stored information attenuating the aversive state and the behaviors it normally promotes.

A second group of researchers focuses on evidence that output from the amygdala modulates ("strengthens") long-term memory storage in various brain regions, and that it directly produces specific aversive behaviors. These workers suggest that amygdala lesions or inactivations eliminate modulation and impair an animal's ability to perform these behaviors rather than affecting the memory itself or an affective state.

The hypothesis underlying the present work is that neuro-plastic changes in the amygdala and related structures store information about aversive associations acquired during aversive conditioning procedures. Subsequent activation of this amygdala-mediated representation leads to the production of an array of unobservable, internal responses that comprise an aversive affective state. The affective state underlies the promotion of various overt behaviors produced by various brain regions. A subset of the internal responses modulates memories in various brain regions. These ideas are supported with evidence from experiments using immunohistochemical assessment of amygdala activation and measurements of competing behaviors and conditioned memory modulation during temporary inactivation of the amygdala.

The first manuscript (submitted to *Behavioural Brain Research*) describes an investigation of amygdala c-Fos protein labeling following aversive unconditioned (shock) and conditioned (contextual cues) stimulus presentation. This was done to examine the

relationship of one measure of amygdala activity to the occurrence of freezing. In the first experiment rats that were shocked and switched to a neutral compartment froze less than rats that stayed in the shock compartment. Notwithstanding these differences in observed freezing, levels of c-Fos expression in both groups were elevated to the same degree in the central and lateral amygdala regions, compared to non-shocked controls. In the second experiment, rats learned to associate one shuttle box compartment with shock and a second, distinct compartment with no shock. Subsequently, freezing was elevated during exposure to both compartments (although less so for the no-shock compartment), but only re-exposure to the shock compartment elevated amygdala c-Fos expression.

These experiments demonstrate for the first time that a population of neurons in the amygdala is activated upon exposure to unconditioned or conditioned aversive stimulation but is unrelated to observed freezing. The findings suggest that the form of amygdala activation measured is more closely related to exposure to unconditioned or conditioned aversive stimuli than it is to observed freezing. The findings are consistent with the hypothesis that amygdala activity may mediate the production of an aversive affective state ("fear") but not the direct production of freezing.

In manuscript two (submitted to *Learning and Memory*) the issue of whether amygdala inactivations disrupt performance of specific behaviors was examined by temporarily inactivating the structure before shock-training or testing and measuring two incompatible behaviors during the test. Freezing (the suppression of behavior) was measured when the rats were confined in the shock-paired compartment; place avoidance (requiring the initiation of behavior) was measured when the rats were allowed to move between the shock-paired compartment and a neutral compartment.

Pre-training inactivation attenuated freezing and eliminated place avoidance. Pre-testing inactivation eliminated both behaviors. These novel findings show that inactivation of the amygdala blocks two incompatible behaviors. Since freezing requires suppression of movement and place avoidance requires its initiation, these effects cannot be attributed to a deficit in the ability to inhibit or initiate behavior. Rather, the findings are consistent with the hypothesis that inactivation of the amygdala disrupted a process required for the production

of an aversive affective state which promotes both freezing and place avoidance depending on environmental constraints during exposure to conditioned aversive stimuli.

The third manuscript (submitted to *Behavioral Neuroscience*) used the conditioned memory modulation effect to examine the idea that the amygdala mediates the production of conditioned internal responses upon exposure to conditioned aversive stimuli. Memory modulation is known to be mediated by the amygdala and certain physiological responses that produce it have been identified. Blockade of conditioned memory modulation by inactivation of the amygdala would suggest that the amygdala is required for the production of these internal responses.

To test this idea rats were exposed to shock in a compartment. Subsequently they were exposed to the aversive conditioned stimuli in the compartment (with no shock) immediately after training on an appetitive radial maze task. This treatment improved performance of the appetitive task, a demonstration of conditioned memory modulation. Inactivation of the amygdala before post-training exposure to the conditioned aversive stimuli eliminated the improvement. It also eliminated both freezing and place avoidance that were measured during post-training exposure to the conditioned aversive stimuli.

This is a novel finding in that it shows that temporary inactivation of the amygdala specifically during exposure to an aversive conditioned stimulus blocked memory modulation, suggesting that the amygdala mediates the production of unobservable, conditioned, physiological internal responses that modulate memories. The finding that amygdala inactivation also disrupted expression of overt behaviors (freezing and place avoidance) suggests that this structure is also involved with their production.

Together with the evidence in the other manuscripts, this suggests that the amygdala may be required for the production of the internal responses that constitute an affective state, and that this state may promote the expression of various overt behaviors. The evidence is also consistent with the hypothesis that the amygdala is critical for the mnemonic representation of the association that produces these conditioned internal responses.

Contributions of Authors

Each of the three manuscripts in this thesis has two authors: the candidate and his

advisor. Their respective contributions to these papers are as follows. The experimental approach to the theoretical issues in Manuscript 1 and the experimental design used were developed independently by the candidate. The idea, in Manuscript 2, that the measurement of two competing behaviors can be used to distinguish between the behavioral production and memory storage hypotheses of amygdala function was developed through discussions between the two authors. In Manuscript 3, the idea that conditioned memory modulation results from an array of internal conditioned responses was suggested by White and McDonald (2002) and developed through extensive discussions between the two authors. The specific experimental designs used in Manuscripts 2 and 3 were developed independently by the candidate. All behavioral testing, histology, data analysis, and writing of the manuscripts was done by the candidate. The candidate's advisor edited several drafts of each manuscript.

Introduction

The purpose of this thesis was to examine the role of the amygdala complex in the mediation of conditioned behaviors. The experimental procedures were designed to obtain measures of several behaviors from the same animals following exposure to unconditioned or conditioned aversive stimuli. Examining the relationship among these behaviors led to inferences about the role of internal responses (sometimes called "fear") in producing the observed behaviors. The experiments also provided information about the amygdala's involvement in aversive conditioning. To introduce this topic, a historical perspective is provided, followed by a discussion of several theoretical models used to infer fearful affective states (the topic of this thesis) and the role of the amygdala in these models.

I. Historical perspective

Early Greeks defined emotions based on body temperature, perspiration (Heraclitus, BC 500), and blood aeration (Diogenes of Apollonia, BC 460). Hippocrates (BC 460; as found in Coar, 1982) postulated that the brain was partly responsible for conscious life and that emotional states were characterized by brain temperature, moisture, and aridity. For Hippocrates, fear arose from the flow of bile resulting in an overheating of the brain.

Aristotle (as translated by Fortenbaugh, 2002) also appears to have suggested that fear could be defined in terms of measurable physiological and behavioral changes. Aristotle's concept of physiology was based on the flow of pneuma throughout the body. Pneuma were located in the heart and contained the source of vital energy responsible for the movement of the limbs. Pneuma also influenced the blood which in turn influenced the mental state of the organism. Fear was the product of cold blood and a redistribution of vital heat due to the motion of blood from the top to the bottom, and from the outside to the inside of the body.

Aristotle also postulated that alterations in pneuma were directly responsible for observable behavioral changes (Fortenbaugh, 2002). A trembling voice was due to spasmodic heart beat as the redistribution of heat occured from the top of the body to the bottom; a shrilling voice was due to a decrease of air flow which was a consequence of heat withdrawal from the top. Thirst and excretion were due to excessive heating of the stomach and excess of heat in lower viscera. Therefore, for Aristotle, changes in the flow of pneuma mediated

an emotional state and the corresponding behavioral changes.

As translated from Plato's works (BC 384 - 322; Zeyl, 2000), the causes of pleasure and pain (emotions) were understood as rising from a disturbance (environmental) that is passed on in a chain reaction (cycle) with parts earlier in the chain affecting others in the same way as they were affected by the initial disturbance. The chain reactions produced by a disturbance were hypothesized to be transmitted via acid and briny phlegms or any bitter and bilious humors (Zeyl, 2000). These humors were thought to affect the mortal soul which was located in the chest and contained the seat of emotion. Therefore, emotions arose from environmental disturbances transmitted to the mortal soul via humors.

Plotinus (204 - 270; as translated by Mackenna, 1991) proposed that passions (emotions) begin with perception and understanding of one's own sensations or disturbances and those of others. These sensations are then transmitted via heated bile or blood which reaches the soul and sets in motion a universal soul activity (Mackenna, 1991). When changes in soul activity occur, the body is perturbed, awareness follows and associates the perturbation with ongoing thoughts, and an emotion is felt. The soul then uses the body as an instrument to perform actions.

Locke (1632 - 1704; reprinted 1979) described pain and pleasure as being derived from both sensation (external) and reflection (internal). While Locke did not explicitly state whether humors or blood were responsible for transmission of the emotions, he suggested that thoughts or perceptions of the mind may be accompanied by pleasure and pain. He postulated that emotions cannot be described nor the thoughts which produce them be defined; rather, understanding of the emotions comes with experience (Locke, 1979). Ideas of love and hatred were dispositions of the mind, however caused. According to Locke, "Fear is an uneasiness of the Mind, upon the thought of future Evil likely to befal (*sic*) us" (p. 231 Locke, 1979). This suggests that Locke ascribed the mediation of emotions to a learning process. He also suggested that the passions (emotions) operate on the body, and cause various changes in it. Therefore, modes of pleasure and pain result from the mind and produce observable changes in the body.

According to the works of Descartes (translated in Halden and Ross, 1967) the

passions (emotions) were perceptions of the mind that arose from external impulses. Descartes postulated that sensory organs transmit information to the pineal gland, where the body joins the mind. This information is transmitted via movement of the animal spirits, a fluid matter. Once a sensation is transmitted to the pineal gland, it can affect the soul. Descartes defined the passions as being caused, maintained, and strengthened by movement of the animal spirits (Halden and Ross, 1967). This movement prepares the body for action. Therefore, if fear results from movement of the animal spirits, the soul will prepare the body to flee.

Hume (1711-1776; reprinted 2000) argued that the passions (perceptions, sensations, or emotions of the souls) have an essentially physical cause. The productive passions arose from natural impulse or instinct, which provided a deep-seated force for human action. Hume argued that the passions are natural instincts derived from the original constitution of the human mind.

One aspect of Hume's ideas (Hume, 2000) was that emotions, on the one hand, and volitions and actions, on the other, are just as regularly and consistently causally related as a wide range of phenomena in the natural world. Hume argued that the prospect of pain or pleasure arouses a desire to modify the circumstances in which these emotions are experienced. These prospects arouse desires to bring about the avoidance of pain or the acquisition of pleasure. Hume also suggested that the emotions interact with reasoning which enables specific courses of action likely to bring about a given end. Whether or not particular information (reasoning) is used depends on particular desires or passions. Hume proposed that the passions determine choices. Reason informs the passions of the existence and nature of things and the steps to be taken if something is to be obtained or avoided.

Kant (1724 - 1804; reprinted 1997) suggested that external stimuli alone never cause an action but rather actions are based on a combination of external stimuli and internal motives (emotions). Humans have lower faculties (emotions) that serve as impulses and can become impelling causes. External stimuli are viewed as an insufficient impetus for action. As argued by Kant, the emotions provided a required impetus that makes actions occur. Alterations of the soul or mind occur with corresponding alterations of the body. Kant

suggested that desires produce motions in the body when they occur in competition with intentions (e.g., when someone is frightened and wants to run away but from fear, is not able to move).

Kant also proposed that nerves were affected by external stimuli and through the nerves, sensation occurs in the soul according to the faculty of pleasure and displeasure whereby the whole body is set into motion (Kant, 1997). Without the faculties of pleasure and displeasure, rational beings would cognize objects without being moved by them. Therefore, one basic power of the soul was to produce alterations in movement. These alterations in movement arose from nerve activity effecting the soul.

Darwin (1872) suggested that when the sensory systems are strongly activated, nerve forces are generated and transmitted in certain directions depending on the connection of the nerve-cells. This excessive "neural transmission", according to Darwin, would reflect the emotional experience and result in the expression of various behaviors (Darwin, 1872). These behaviors would be expressed to gratify certain sensations (emotions). Whenever a specific state of mind was induced by the neural transmission, there would be a tendency for the same movements to occur. Darwin also suggested (Darwin, 1872) that certain observable actions normally associated with particular states of mind could be suppressed through the will. However, suppression of one action would require other movements which would still be expressive of the emotional experience.

William James (1884) described affective states or emotional experiences as resulting from a set of behaviors or other peripheral reflexes elicited by some "exciting object". As described by James (1890), the psychological experience of fear results from changes in observable behaviors produced directly by threatening stimuli. Feedback from changes in these observable behaviors leads to the fearful affective state.

Freud also appears to have been a proponent of the idea that fearful, affective states arise from observable behaviors (Freud, 1936). Fear is used here where Freud used the term anxiety. Freud argued that a painful stimulus produces a set of observable defensive reactions (behaviors). These observable behaviors are elicited directly by the painful stimulus, and on later occasions, by the stimuli that surrounded the painful event. According to this

hypothesis, these behavioral defensive reactions lead to a state of heightened tension (anxiety), considered to be equivalent to the psychological experience of fear.

In contrast to James and Freud, Cannon and Bard (Cannon, 1927) suggested that a fearful affective state results from a set of unobservable processes (mental processes) elicited directly by the perception of aversive events. An aversive environmental event directly activates a set of central, neurological processes resulting in the subjective experience of an emotion (fear). Behaviors then emerge based on these central, neurological processes.

The idea that affective states arise from central processes was also argued by Hebb (1946). Hebb proposed that a fearful psychological state results from the disruption of certain "organized cerebral activities" and that "autonomous" central processes are directly activated by the perception of aversive environmental events (Hebb, 1946). The central cerebral activity is not only responsible for the subjective experience of fear but also underlies the expression of behaviors associated with fear (e.g., escape, freezing, vocalizations).

The present thesis contains elements from the writings of these early authors. Affective states may result from a set of physiological, central or internal responses. These internal responses result from the perception of aversive environmental events or activation of neural representations of these aversive events. These internal responses may include neurotransmitter release, neurohormonal release, and a variety of additional autonomic responses. In addition, these internal responses result in the expression of different overt behaviors suggesting that internal responses reflecting emotional states can be inferred through the measurement of different overt behaviors.

II. Contemporary theories

A. Learned Behaviors

Mowrer (Mowrer, 1939; 1947; Mowrer and Lamoreaux, 1946) argued that affective states (in particular, fear) can be inferred by observing learned behaviors. Mowrer (1947) suggested that a fearful affective state is produced by stimuli associated with threatening or aversive situations. During exposure to these stimuli, the affective state is elicited regardless of the environmental configuration that contains the stimuli. Mowrer argued that the affective state prepares ("... provides the *incentive* or drive..." p. 29 Mowrer and Lamoreaux, 1946)

the organism in advance of threat so that when the organism experiences the affective state, adaptive behaviors (such as avoidance) emerge to interact with the specific environmental configurations. The behaviors are emitted instrumentally to prevent injury (Mowrer, 1947). Observation of these learned behaviors allows one to infer the affective state of the organism.

A similar idea was suggested by Miller (1948). Miller proposed that fear is elicited following exposure to a variety of stimuli associated with threat. As argued by Miller (1948), fear is composed of a set of internal responses ("... central impulses which travel from the thalamus to sensory areas of the cortex..."; p. 98 Miller, 1948) and these responses serve as a drive or a stimulus. Miller (1948) further argued that when these internal responses are present, random behaviors emerge. When one of these behaviors results in elimination of the internal responses, this reinforces the behavior. Miller concluded by stating that when fear is learned in a particular situation, all of the behaviors which have been emitted to reduce fear in other situations, as well as unlearned behaviors, will be transferred to that situation (Miller, 1948). Therefore, it is the internal responses that serve as both a drive and a stimulus, rather than the specific situation, that elicit observable behaviors.

The view that a fearful affective state functions as a drive was further argued by Brown and Jacobs (1949). They agreed with Miller's notion that fear-reduction can reinforce the acquisition of new behaviors. In one study (Brown and Jacobs, 1949), rats were trained to jump over a barrier during presentation of a stimulus (tone) that had previously been paired with shock. They argued that the tone produced an aversive affective state that motivated the acquisition of the avoidance (jumping) behavior (Brown and Jacobs, 1949). The behavior was reinforced by a reduction in the aversive affective state. Therefore, observation of these learned behaviors served as an indirect assessment of the aversive affective state.

The hypotheses above (Mowrer, Miller, and Brown and Jacobs) are reflected in the Lazarus-Schacter theory of emotion (Lazarus, 1991). This theory states that emotional experiences can be inferred by an observer from behavior. During an encounter with a threatening environmental situation, a fearful affective state is produced. Specific behaviors are then emitted as a way of interacting with the immediate environmental configuration. The behaviors reduce the affective state and can be used by an observer to infer its nature and

intensity. These hypotheses (Brown and Jacobs, 1949; Lazarus, 1991; Miller, 1948; Mowrer, 1939; 1947; Mowrer and Lamoreaux, 1946) center on the argument that the occurrence of "instrumental" or learned behaviors provides an indirect assessment of an aversive affective state elicited by aversive events.

B. Unlearned behaviors

Brown, et al. (1951) hypothesized that aversive affective states could also be inferred by measuring the amplification of unlearned or innate behaviors. They (Brown, Kalish, and Farber, 1951) reported that an amplified startle reaction was observed in response to a noise following presentation of a tone previously paired with shock. They argued that the innate startle reaction was intensified by a learned or conditioned aversive affective state ("central state") produced by the shock-paired tone. Measurement of the elevated innate or unlearned reactions (e.g., startle response, freezing) provided an indirect assessment of the aversive affective state.

The idea of measuring the amplification of unlearned behaviors to assess affective states was elaborated on by Davis (1992; 1997; 2000), who also argued that a "central state of fear" can be inferred from amplified innate reactions (e.g., startle, freezing). Davis proposed that this state acts as an intervening variable between stimulus input and behavioral output. Painful or aversive environmental stimuli produce a central state of fear which amplifies physiological and behavioral responses such as freezing and startle. He hypothesized that, through classical conditioning, stimuli paired with painful or aversive events produce a similar central state leading to potentiation of the same responses. The amplified innate behaviors or reactions can then be used to assess the strength of the conditioned central state of fear.

III. Summary

These descriptions suggest that a fearful affective state results from a set of physiological, central processes referred to as internal responses in the present thesis. These internal responses result from the perception of aversive environmental events or activation of learned neural representations of these aversive events. These internal responses are hypothesized to result in different overt behaviors; the form of which depends on information

concerning the environmental configuration.

The relationship between internal responses and overt behaviors is central to the present thesis. Internal responses are unobservable physiological responses produced by the perception of aversive environmental events or that result from the activation of neural representations of these events (Miller, 1948; Mowrer and Lamoreaux, 1946). These responses may take the form of neurotransmitter or neurohormonal release and contribute to what has historically been referred to as a psychological emotional experience, or affective state (Hippocrates - Coar, 1982; Aristotle - Fortenbaugh, 2002; Plato - Zeyl, 2000). Internal responses may be similar to what others have referred to as central activation (Cannon, 1927; Freud, 1936) or central processes (Hebb, 1946; James, 1890) and some combination of internal responses comprises an unobservable affective (fearful) state (Brown, Kalish, and Farber, 1951; Davis, 1992; 1997; LeDoux, 1998; 2000; McAllister and McAllister, 1971). Direct measurement of the entire array of internal responses, if possible, would provide a direct assessment of the affective state experienced by the organism.

Overt behaviors are changes in motor patterns quantified by direct observation. These behaviors may be part of the organisms' innate behavioral repertoire (Bolles, 1970; 1976) but may also include learned behaviors emitted by the organism to reduce exposure to an aversive situation (Brown and Jacobs, 1949; Miller, 1948). This second form of overt behavior is based on an ability to interact flexibly with a variety of environmental configurations. Overt behaviors are, in most cases, observed during exposure to conditioned aversive stimuli and may reflect a combination of internal responses and information concerning the environmental configuration.

IV. Current Literature

A. Experimental Study of Fear

Neutral stimuli can gain the ability to alter the behavior of an organism through the formation of associations with biologically important (unconditioned) stimuli. As translated into Pavlov's terminology (Pavlov, 1927), an unconditioned stimulus (US) elicits various unconditioned responses (UR). The US may occur in the presence of neutral cues (CS) that do not themselves elicit similar responses. Following repeated occurrences of the CS with

the US, the CS begins to evoke conditioned responses (CR) when presented alone.

When painful or aversive events (US) have been paired with a CS, the CS elicits a CR when presented alone. This kind of Pavlovian conditioning is usually referred to as fear conditioning. This form of learning proceeds optimally when a cue such as a tone, light, or an experimental context (the CS) is presented with an aversive event (usually a shock; the US). Ordinarily, only a few pairings of the CS and US are necessary for conditioning to occur so that when the CS is presented in the absence of the US, a variety of behavioral changes, usually involving some combination of freezing and escape, is observed (see for example Amorapanth, LeDoux, and Nader, 2000; Antoniadis and McDonald, 1999; Fendt, 2001; Holahan and White, 2002; Lee, Choi, Brown, and Kim, 2001). Expression of these behaviors may be sensitive to the environmental configuration in which the CS is presented (for reviews see Bouton, 1993; Bouton and Bolles, 1980; Davis, 2000; Fanselow, 2000; LeDoux, 1993; 1995; 2000; McAllister and McAllister, 1971; Rescorla, 1988; Rescorla and Solomon, 1967).

In addition to observable behavioral changes, the US (shock) initiates an array of internal responses (UR) such as neurotransmitter, neurohormonal, and autonomic changes (Cannon, 1927; Davis, 2000; Fanselow and Gale, 2003; Freud, 1936; Hebb, 1946; James, 1890; LeDoux, 2000; Maren, 2001a; White and McDonald, 2002). Upon re-exposure to the CS, a similar array of internal responses (CR) is thought to be produced (Cannon, 1927; Davis, 2000; Fanselow and Gale, 2003; Freud, 1936; Hebb, 1946; James, 1890; LeDoux, 2000; Maren, 2001a; White and McDonald, 2002). The central hypothesis is that the array of internal responses that constitute the UR and CR are the psychological affective state. A purpose of the present thesis is to examine the role of the amygdala in the mediation of observable behavioral changes during aversive conditioning and the process of inferring the existence of internal responses from these behaviors.

B. Freezing

1. Background

One overt behavior that is elevated by threatening or aversive events is a skeletal withdrawal behavior referred to as crouching or freezing (Brown and Jacobs, 1949). This

behavior has been described as a stereotypical, species-specific reaction to threatening stimuli (Bolles, 1970; 1976). The behavior is operationalized as a cessation of motor activity including whisker and nose movements (Bolles and Collier, 1976) and sitting rigidly motionless (Bindra and Anchel, 1963) except for movement necessitated by respiration (Fanselow, 1980).

Initial observations of freezing during aversive conditioning procedures were thought to be the result of shock (the US) administration. Shock would elicit freezing (the UR) and cues paired with shock would subsequently elicit the same behavior (CR). However, there is compelling evidence suggesting that a majority of freezing is elicited exclusively by cues paired with shock (the CS) and that very little freezing is elicited by the US itself. This suggests that freezing does not necessarily follow the conventions of Pavlovian conditioning.

In one report, mice displayed less activity when they were tested in an open field apparatus constructed with a rod floor that permitted the administration of foot shocks (Baron, 1964). Following foot shock, the mean number of squares crossed in the open field decreased from the preshock level while the number of inactive periods increased. The author interpreted the elevation in inactive periods as a direct result of shock administration. Blanchard, *et al.* (1968) reported the presence of a stereotyped crouching posture (freezing) following foot shock. This behavior in shocked rats compared to nonshocked rats was the predominant difference in behavior that lasted up to two hours following termination of the foot shock. The authors interpreted this difference as resulting from foot shock. In another study (Blanchard and Blanchard, 1969a), a group of rats received multiple shocks from a mobile shock prod. They subsequently crossed fewer squares and spent more time freezing during the inter-trial intervals than a no shock group. In a further study (Blanchard and Blanchard, 1969b) it was reported that the time spent freezing was a direct function of the shock intensity. Freezing in both of these studies (Blanchard and Blanchard, 1969a; 1969b) was interpreted to result from shock administration.

When rats were shocked in one arm of a 3 arm y-maze, there was a marked reduction in approach to the shock arm and in exploration of the maze (Kumar, 1970). Kumar concluded that the depressed activity was a reaction to the shock US. In another study

(Brener and Goesling, 1970) rats were trained to avoid shock by moving 1 inch in any direction or by remaining immobile (freeze) during a signal predictive of shock. The immobile group avoided significantly more shock presentations than the active group. The authors hypothesized that the superior performance of the immobile group was due to the freezing elicited by the shock.

A number of studies (Blanchard and Blanchard, 1969b; Bolles and Collier, 1976; Fanselow, 1980) have specifically addressed whether freezing is elicited by the shock US or by cues paired with the US. In these studies (Blanchard and Blanchard, 1969b; Bolles and Collier, 1976; Fanselow, 1980) rats were shocked in one context and immediately tested in the same or a different context. High levels of freezing were observed in the shock context but freezing was reduced in the different context immediately following shock. These results indicated that a major portion of freezing was not a direct consequence of the US but rather that it was due to cues paired with shock.

The idea that freezing is elicited by the CS has gained increasing support (Fanselow, 1980; 1982; 1984; Fanselow, Landeira-Fernandez, DeCola, and Kim, 1994; Kiernan, Westbrook, and Cranney, 1995; Maren, 2001a; Sacchetti, Lorenzini, Baldi, Tassoni, and Bucherelli, 1999). As reported by Bindra and Anchel (1963) rats shocked in one context and tested in that context displayed more immobility (freezing) than rats shocked in the same context but tested in three other distinct contexts. Freezing was found to be a function of contextual discriminability. The authors proposed that the immobility measured in the absence of shock was elicited by the contextual CS (Bindra and Anchel, 1963). This is consistent with the results of a study where rats shocked in one of two boxes (long or short) froze significantly more when they were tested in the shock box than when they were tested in the neutral box (Bolles and Collier, 1976). The authors concluded that the principal factor influencing the change in behavior was the presence of cues previously paired with shock. A similar study (Fanselow, 1980) assessed freezing immediately after shock in the shock context and 24 hours later in the shock-paired context or in a neutral context. Rats tested in the neutral context froze less than rats tested in the shock-paired context. Furthermore, there was no reduction in freezing 24 hours after shock administration when the rats were re-

exposed to the shock-paired context. Fanselow argued that if freezing was a reaction to foot shock, it should diminish during a protracted time period following the shock (Fanselow, 1980). Since this did not happen, it was concluded that freezing was elicited by the CS.

Stimulus pre-exposure studies using the training context as the stimulus, have also provided evidence that freezing is elicited and maintained by cues surrounding foot shock rather than by shock. Blanchard and Blanchard (1968a) observed that post-shock freezing was significantly attenuated by an extended pre-exposure to the shock compartment prior to shock. Another report (Kiernan and Westbrook, 1993) supported the finding that brief pre-exposures to the to-be-shocked context elevated freezing whereas longer pre-exposures diminished the amount of freezing. It was argued that habituation to the contextual cues produced a deficit in the development of a conditioned association between the cues and the shock due to latent inhibition (Chacto and Lubow, 1967; Lubow, 1965; Lubow, Markman, and Allen, 1968; Lubow and Moore, 1959).

Other studies (Blanchard, Fukunaga, and Blanchard, 1976; Fanselow, 1986; Kiernan, Westbrook, and Cranney, 1995) have found that when animals were shocked immediately after placement into a compartment they displayed less freezing when tested in that compartment in the absence of shock than animals that experienced a delay between placement into the compartment and shock. The authors suggested that rats shocked immediately were not able to form an association between the array of contextual cues and shock. Animals shocked after the delay were able to acquire a "spatial" representation of the context and form an association between the apparatus cues and the shock resulting in elevated freezing.

Although the experiments described suggested that a majority of the freezing observed following shock is due to cues paired with shock some freezing may also be produced directly by the shock. Figure 1 in Blanchard and Blanchard (1969b p. 371) and Figure 1 in Bolles and Collier (1976 p. 7) show that rats tested immediately after shock in a neutral context froze for approximately 10 - 15% of the observation time. Table 1 in Fanselow (1980 p. 179) also shows that rats tested immediately after shock in the neutral context displayed more freezing (10 - 14%) than rats that never received foot shock (1%). This suggests that a certain

amount of freezing can occur as a result of shock in the absence of cues predictive of shock.

The evidence reviewed indicates that the majority of freezing observed following footshock is elicited by cues associated with shock rather than by the shock itself. Freezing to a contextual CS is observed only if the animal has had sufficient opportunity to acquire information about the complex cue configuration of the environment before the shock is administered. There is also evidence that some freezing may be produced directly by the shock.

Other USs, such as predators or their odors, have also been associated with elevations in freezing. In one of the original reports (Blanchard and Blanchard, 1971) rats that were confined in a transparent, enclosed compartment exhibited higher levels of freezing in the presence of a cat than a control group. Rats confined in a wire mesh cage and exposed to a cat froze for approximately half of the 15 minute observation period (Canteras, Chiavegatto, Ribeiro do Valle, and Swanson, 1997). When rats were exposed to cat hair, they froze for approximately 30 - 50% of the observation time (Vazdarjanova, Cahill, and McGaugh, 2001). It has also been reported that the odor from fox feces elicits high levels of freezing (Wallace and Rosen, 2000). These increases in freezing are usually interpreted as resulting from the predator odor US.

Studies that used predators or predator odors as the aversive US also contain evidence that freezing may be sensitive to cues associated with aversive events. When rats were provided with an alley that permitted escape from a cat, rats displayed very little freezing and ran away from (avoided) the cat (Blanchard and Blanchard, 1971). If freezing was directly elicited by the cat, the animals would have frozen regardless of the apparatus configuration. Using a cat odor, it was found that rats escaped to and hid under a food hopper that was present in the testing cage (File, Zangrossi, Sanders, and Mabbutt, 1993; Zangrossi and File, 1994). The authors classified this behavior as avoidance and suggested it shows that freezing may be sensitive to the apparatus configuration. Finally, rats exposed to a cat odor or neutral odor displayed similar levels of locomotor activity and rearing in a neutral environment 5 or 35 minutes after the exposure (Zangrossi and File, 1992). If freezing was elicited unconditionally by the cat odor (the US), measures of activity in the neutral environment

would have been lower in the rats exposed to the cat odor as freezing would have persisted in the neutral context and interfered with normal movement.

2. Amygdala and freezing

A number of studies have found that impairing the function of the amygdala interferes with freezing (for review see Davis, 2000; Fendt and Fanselow, 1999; LeDoux, 1993; 1995; 1996; 1998; 2000; Maren, 2001a). As pointed out in the previous section, freezing appears to be maintained mainly by cues paired with shock. Therefore, many have argued (Fanselow and LeDoux, 1999; LeDoux, 2000; Maren, 1999; 2001a) that reductions in freezing following an amygdala lesion reflects the elimination of a neural representation of an association between the CS and US.

Radiofrequency or electrolytic lesions which produce localized and well-defined lesions, have been used to examine the function of the amygdala in the occurrence of freezing. Early evidence (Blanchard and Blanchard, 1972) found that large radiofrequency amygdala lesions and lesions restricted to the medial amygdala (MeA) reduced freezing when rats were placed into a compartment where they had previously been shocked. Similar lesions reduced the duration of freezing in the presence of a cat (Blanchard and Blanchard, 1972; Blanchard and Takahashi, 1988) but did not affect locomotor activity (Blanchard and Takahashi, 1988). Furthermore, large electrolytic lesions (Kim, Rison, and Fanselow, 1993; Phillips and LeDoux, 1992) of the amygdala complex eliminated freezing when rats were placed into a compartment where they had previously been shocked.

Lesions restricted to specific amygdala subregions also reduce freezing in the presence of shock-paired cues. Electrolytic lesions restricted to the lateral amygdala (LA) (Amorapanth, LeDoux, and Nader, 2000; Holahan and White, 2002; LeDoux, Cicchetti, Xagoraris, and Romanski, 1990; LeDoux, Iwata, Cicchetti, and Reiss, 1988; Nader, Majidishad, Amorapanth, and LeDoux, 2001) reduced freezing during presentation of a shock-paired tone. Electrolytic lesions of the central amygdala (CeA) or the basolateral amygdala (BLA) (Campeau and Davis, 1995) reduced freezing to a tone or light previously paired with foot shock. Electrolytic lesions of the BLA, CeA, or LA attenuated freezing to both a shock-conditioned context and tone (Holahan and White, 2002) while MeA lesions

reduced freezing to the context but not the tone.

Neurotoxic amygdala lesions that disrupt cellular function and produce little damage to fibers of passage (Brace, Latimer, and Winn, 1997) have also been shown to eliminate freezing in the presence of shock-paired cues. Pretraining NMDA lesions centered on the BLA (Antoniadis and McDonald, 2000; Cahill, Vazdarjanova, and Setlow, 2000; Maren, 1998; 1999; 2001b; Maren, Aharonov, Stote, and Fanselow, 1996; Vazdarjanova and McGaugh, 1998) blocked freezing when rats were exposed to shock-conditioned cues. Ibotenic acid lesions of the CeA or NMDA lesions of the BLA (Campeau and Davis, 1995) reduced freezing to a tone or light previously paired with shock. Lesions centered on the LA or CeA in one hemisphere combined with total electrolytic lesions of the amygdala complex in the other hemisphere reduced freezing to a shock-conditioned context and tone (Goosens and Maren, 2001).

Postttraining lesions of the amygdala also reduce freezing in the presence of shock-paired cues. Electrolytic lesions of the CeA made 6 or 30 days after shock-training (Kim and Davis, 1993) or NMDA-induced lesions of the BLA (Lee, Walker, and Davis, 1996) made 6 or 30 days after shock-training blocked freezing in the presence of a shock-paired tone. NMDA lesions centered on the BLA made 1, 14, or 28 days after tone-shock pairings (Maren, Aharonov, Stote, and Fanselow, 1996) or context shock pairings (Maren, 1998) abolished freezing to both the conditioned tone and contextual cues at all training-to-lesion intervals. NMDA lesions centered on the BLA made 1 or 15 days after training reduced freezing to a shock-conditioned olfactory CS and the training context (Cousens and Otto, 1998).

Intra-amygdala injections of various drugs that interfere with normal amygdala function also reduce freezing during exposure to shock-paired cues. Infusion of an NMDA antagonist (AP-5) into the BLA before context-shock pairings (Fanselow and Kim, 1994) or tone-shock pairings (Campeau, Miserendino, and Davis, 1992; Miserendino, Sananes, Melia, and Davis, 1990) reduced freezing during re-exposure to the CS. The interpretation of these results was that temporary blockade of NMDA receptors in the amygdala blocked a plasticity-related process (Keith and Rudy, 1990). However, infusion of AP-5 into the BLA

immediately before testing blocked freezing to a shock-conditioned context (Maren, Aharonov, Stote, and Fanselow, 1996), a shock-conditioned tone or context (Lee, Choi, Brown, and Kim, 2001), or a shock-conditioned light (Fendt, 2001). The findings that NDMA receptor antagonists block the expression of freezing have been explained as a disruption of normal synaptic transmission (Keith and Rudy, 1990; LeDoux, 1996) rather than a specific effect on plasticity.

Lidocaine injections into the BLA/CeA (Helmstetter, 1992) attenuated freezing when the injection was given immediately before testing but had less effect when administered before CS-US pairings. Pretraining injections of diazepam into the BLA and to a lesser extent, the CeA, reduced the amount of immediate postshock freezing compared to a vehicle injected control group (Helmstetter, 1993). Rats with cannulas aimed at the BLA (Helmstetter and Bellgowan, 1994) received a $GABA_A$ agonist (muscimol) injection before CS-US pairings or before testing. Intra-amygdala injections immediately before the test completely eliminated freezing whereas injections made before training resulted in a smaller attenuation of freezing during the test. It has also been reported that muscimol infusion into the LA/BLA before training or testing completely eliminated freezing (Muller, Corodimas, Fridel, and LeDoux, 1997). Injections of muscimol into the LA/BLA before tone-shock pairings dose-dependently reduced freezing during re-exposure to the tone CS (Wilensky, Schafe, and LeDoux, 1999; 2000). While pretesting intra-amygdala injections of neural inhibitors consistently eliminate freezing, pretraining injections have less consistent effects.

3. Summary of amygdala and freezing

It has been argued that reductions in freezing may be due to lesion- or inactivation-produced impairments in the rats' ability to produce the freezing behavior (Cahill, McGaugh, and Weinberger, 2001; Cahill, Vazdarjanova, and Setlow, 2000; Cahill, Weinberger, Roozendaal, and McGaugh, 1999; Vazdarjanova, Cahill, and McGaugh, 2001). Alternatively, the amygdala may mediate a mnemonic process that promotes freezing in the presence of an aversive CS (for example see Maren, 1999; 2001a; 2001b). It may also be the case that such lesions disrupt both the innate ability to respond to aversive stimuli and the representation of the learned association.

C. Avoidance

1. Background

Avoidance occurs when an organism engages in some behavior that minimizes exposure to a cue or set of cues predictive of an aversive event (Olton, 1973; Wadenberg and Hicks, 1999). Avoidance can be either active or passive (Blanchard and Blanchard, 1968a; 1968b; 1968c; 1970a; 1970b). Active avoidance (the form studied in the present thesis) is observed when an organism initiates a set of behaviors such as pressing a lever or moving out of a compartment to reduce exposure to aversive cues (Wadenberg and Hicks, 1999). Active avoidance is incompatible with freezing because freezing interferes with the expression of active behaviors.

In the standard passive avoidance procedure, rats are placed in a brightly lit compartment joined to a dark compartment. Normal rats prefer to be in the dark compartment and so move into it. Once inside the dark compartment, they receive a shock. When the rats are placed back into the lit compartment they inhibit their normal behavioral tendency to move from the lit compartment to the dark compartment thereby displaying passive avoidance. Passive avoidance resembles and appears to be compatible with freezing because an animal that freezes inhibits other behavioral tendencies and is therefore not likely to enter an aversive compartment or perform any other behavior. Active avoidance differs from passive avoidance because for active avoidance, the organism must learn or initiate new behaviors to reduce exposure to the aversive cues.

Studies of active avoidance (Sidman, 1962a; 1962b) have found that rats can learn to approach and press a lever during the presentation of a cue paired with foot shock to avoid shock administration. The author also reported that some rats displayed immobility (freezing) and were unable to press the lever. This is consistent with the notion that active avoidance and freezing are incompatible (see also Anisman, 1973; Anisman and Waller, 1972; 1973). In a two compartment shuttle-box apparatus, rats that received foot shock upon entry into one compartment quickly learned to avoid the shock by running to the other compartment (Blanchard and Blanchard, 1968b). This shows how active avoidance behavior functions to remove the animal from aversive stimuli.

Further analysis of active avoidance is consistent with the idea that rats can learn specific behaviors to avoid cues paired with shock. One study (McAllister and McAllister, 1962) demonstrated that forward-conditioning (light-shock) led to superior performance on a hurdle-jumping task than backward-conditioning (shock-light) when the light CS was presented alone. The authors concluded that the avoidance occurred with forward conditioning because the animals learned that the light predicted the occurrence of shock and therefore learned to avoid the shock. In another experiment (Christophersen and Denny, 1967) rats learned to avoid shock by pressing a lever during a tone that predicted the shock. A further report (Keehn, 1967) found that rats learned to run in a stationary wheel during a signal predictive of shock, to avoid presentation of the shock. Both of these experiments provide evidence that specific features of the environment determine the behavior the rats acquire to avoid exposure to aversive stimuli. Blanchard and Blanchard (1969a) found that one week following shock administration through a movable prod rats avoid avoided the prod by moving away from it when it was introduced into the cage. This suggests that avoidance was based on learning an association between the specific cue (prod) and administration of shock. The specific behavior was based on the availability of the escape behavior afforded by the environment.

Avoidance has also been observed during exposure to aversive apparatus cues. Two studies (Goldstein, 1960; McAllister and McAllister, 1962) reported that normal rats that received backward conditioning to a discrete tone emitted avoidance behavior during presentation of the tone in the training context. Since backward conditioning would not lead to the formation of Pavlovian associations involving the tone (Pavlov, 1927), the authors concluded that the contextual cues elicited the avoidance behavior.

The conclusion that contextual cues can elicit active avoidance has been supported by several studies. Miller (1948) reported that rats avoided a shock-paired compartment when given the choice between the shock compartment and an adjacent neutral compartment. It was concluded that the cues in the compartment associated with shock led to the avoidance behavior. Another study examined the behavior of rats that were shocked in either the black or white compartment of a shuttle-box (Campbell and Campbell, 1962). When rats

were placed on the side previously paired with shock in the absence of shock, they actively moved out of the shock-paired compartment. It was hypothesized that the rats learned a relationship between the shock and no-shock compartments and were able to direct their behavior to avoid the shock-paired compartment. In another set of studies (Cimadevilla, Fenton, and Bures, 2000a; 2000b), every time a rat entered a specific area in a circular arena defined only by the surrounding cues they were given shock. The rats subsequently spent less time in and made fewer entries into the shock area.

When rats were placed into a compartment one week after receiving unavoidable shock, the proportion of time spent in a neutral, adjacent compartment increased as a function of the shock intensity used during training (Blanchard and Blanchard, 1968a). Similarly, rats confined and shocked in one arm of a 2-arm y-maze that were subsequently allowed to move between the shock and no-shock arms spent less time in the shock arm as a function of the previous shock intensity (Kumar, 1970).

Avoidance based on contextual cues has also been observed using other aversive USs during training. Rats that received intraperitoneal injections of lithium chloride paired with one set of contextual cues (Sovran, 1994; White and Carr, 1985) avoided those cues and spent more time in a neutral, adjacent context when allowed to move between the two contexts.

This review of the active avoidance literature leads to several conclusions. Rats can learn to engage in active behaviors to avoid a discrete cue that has previously been paired with shock. Rats can also learn to avoid contextual cues (i.e., training compartment) that have previously been paired with an aversive event. As found in several studies (Blanchard and Blanchard, 1970b; Mowrer and Lamoreaux, 1946; Mowrer and Miller, 1942) rats avoided cues paired with shock if they were able to discriminate the shock-paired cues from cues not paired with shock. This type of discrimination can develop while a rat freely explores a to-be-paired context and a neutral context prior to experiencing the aversive event in the paired context (Antoniadis and McDonald, 1999). This discrimination can also develop when the rat experiences the two compartments at different times, experiencing the aversive stimulus in one of them (Sovran, 1994; White and Carr, 1985).

2. Amygdala and avoidance

Active avoidance provides a measure of aversive conditioning that is not based on the ability of an animal to inhibit behavior. Rather, the animal must initiate or perform certain behaviors to move way from or reduce its exposure to the aversive cues. While a number of studies have shown that amygdala lesions interfere with the expression of passive or inhibitory avoidance (Bermúdez-Rattoni, Introini-Collison, Coleman-Mesches, and McGaugh, 1997; Dunn and Everitt, 1988; Harris and Westbrook, 1995; Parent, Avila, and McGaugh, 1995; Parent, Quirarte, Cahill, and McGaugh, 1995; Parent, Tomaz, and McGaugh, 1992; Parent, West, and McGaugh, 1994), amygdala involvement in active avoidance has been less studied.

Two studies reported that electrolytic (Gaston and Freed, 1969) or NMDA (Antoniadis and McDonald, 2000) lesions of the amygdala complex impaired place avoidance of a shock conditioned context. In Gaston and Freed, the amygdala lesioned animals spent significantly more time in the white compartment whether it was paired or not paired with shock. This suggests that the amygdala lesions may have resulted in some other deficiencies rather than a specific impairment of avoidance. In another study (Blanchard and Blanchard, 1972), rats with lesions of the entire amygdala complex, or lesions restricted to the MeA/CeA, showed reduced avoidance of a cat and a CS previously paired with footshock. Electrolytic or NMDA lesions of the BLA but not CeA disrupted avoidance of a compartment where rats had previously received lithium chloride injections (Sovran, 1994).

In another study (Killcross, Robbins, and Everitt, 1997), rats were trained on a variable interval schedule to press a bar for food. They were then given either sham lesions or CeA or BLA neurotoxic lesions and after recovery, placed back into the chambers and allowed to press for food. During this second stage, if the rats pressed one lever during presentation of an auditory CS, a shock followed. Pressing on the other lever did not result in shock. BLA but not CeA lesions impaired the animals' ability to avoid pressing the lever that resulted in shock. CeA but not BLA lesions blocked suppression of bar pressing during presentation of the CS. This suggests that BLA lesions specifically blocked active avoidance.

Rats with electrolytic lesions of the BLA, LA, or CeA were trained with Pavlovian presentations of a tone and shock in a compartment (Amorapanth, LeDoux, and Nader,

2000). CeA or LA lesions blocked freezing to the tone while lesions of the BLA or LA impaired escape from the tone. This suggests that BLA lesions specifically blocked the active escape behavior.

The finding that BLA lesions block active avoidance behaviors is not consistent, particularly when the lesions spare the CeA or MeA. Ibotenic acid lesions of the LA/BLA that spared the CeA did not block acquisition of a task in which rats avoided shock by crossing a hurdle within 5 seconds of being placed into the apparatus (Jellestad and Cabrera, 1986). Quinolinic acid lesions restricted to the BLA (Selden, Everitt, Jarrard, and Robbins, 1991) did not reduce avoidance of a compartment previously paired with shock. Ibotenic acid lesions restricted to the BLA (Ambrogi Lorenzini, Bucherelli, Giachetti, Mugnai, and Tassoni, 1991) did not disrupt active avoidance (exit latency; see Figs. 3 and 4, Pp. 767 and 768) from a compartment previously paired with shock but did disrupt passive avoidance (lesions decreased the latency to enter the compartment paired with foot shock). Furthermore, NMDA lesions of the BLA that did not damage the CeA (Vazdarjanova and McGaugh, 1998) failed to produce deficits in avoidance of the shock-paired arm in a 3 arm maze. Similarly, electrolytic lesions restricted to the BLA did not reduce place avoidance behavior (Holahan and White, 2002). These findings raise the question of whether the BLA alone mediates active avoidance.

Other workers have found that when damage included the CeA, active avoidance was impaired. Jellestad and Cabrera (1986) found that ibotenic acid lesions restricted to the BLA did not reduce active avoidance (see above), but when the lesions included the BLA and CeA, there was an increase in the number of trials required to reach avoidance criterion during training and testing. Holahan and White (2002) found that CeA or MeA lesions reduced avoidance of a shock-paired compartment. As permanent electrolytic lesions were used in that study, it is difficult to determine if the deficit was based on the acquisition or expression of the behavior.

Lesions of the CeA also block active, shock-avoidance behaviors in rabbits. Rabbits with electrolytic lesions centered on the BLA that spread beyond the boundaries of the BLA to the LA and CeA (Poremba and Gabriel, 1997) required more training sessions (i.e., tone-

shock pairings) to attain the avoidance criterion than sham lesioned animals. When the amount of tissue damage was compared to the avoidance deficit, it was found that more damage in the CeA but not BLA was significantly correlated with a greater avoidance deficit. The finding that CeA lesions block active avoidance was replicated when a separate group of rabbits with CeA lesions was tested on the same task (Smith, Monteverde, Schwartz, Freeman, and Gabriel, 2001) and active avoidance was blocked.

3. Summary of amygdala and avoidance

The amygdala appears to be involved in active avoidance but this involvement does not appear to be restricted to one subregion. The fact that rats perform the active avoidance behavior for the first time on the test day is often interpreted as suggesting that exposure to the CS activated an affective state or internal responses (Miller, 1948; Mowrer, 1947). The rats generate the avoidance behaviors to move away from the aversive CS and reduce the internal responses (Miller, 1948; Mowrer, 1947; Rescorla and Solomon, 1967). An amygdala lesion might eliminate the production of this aversive affective state and reduce the avoidance behaviors. Alternatively, amygdala lesions might impair the ability to initiate active behaviors. To examine these issues, experiments reported in the present thesis measured place avoidance with temporary inactivation of the amygdala complex during training or testing.

D. Memory modulation

1. Background

An early experiment by Lashley (1917) found that rats injected with strychnine before each training trial required half as many trials to learn a maze task as water injected controls. Lashley hypothesized that the enhancement of learning depended on simultaneous activation of afferent pathways by the drug and by performance of the maze task. This study was replicated some years later (McGaugh and Petrinovich, 1959) and the authors provided a similar interpretation of the results. They suggested that strychnine may reduce synaptic resistance and "facilitate learning by increasing the efficiency of transmission in the central nervous system." (pg 102 McGaugh and Petrinovich, 1959).

The enhanced learning produced by the strychnine injections (Lashley, 1917; McGaugh and Petrinovich, 1959) may have been based on an enhancement of memory

consolidation. Consolidation is the naturally occurring process whereby a recently acquired memory becomes permanent over time. In 1900, Müller and Pilzecker (as described in Lechner, Squire, and Byrne, 1999) proposed that a temporary reverberation of neural activity representing the memory or "trace" of an event persists after the event occurs and that consolidation of the trace takes place during this period. The reverberating activity described by Müller and Pilzecker may be enhanced by the strychnine injections which would have the effect of enhancing consolidation of the memory trace.

To examine whether strychnine affects reverberating neural activity and hence a consolidation process, McGaugh, *et al.* (1962) administered strychnine immediately after training. The rationale was that if neural activity reverberated following acquisition of information then stimulants that enhanced this activity would improve consolidation and therefore, retention of the event (McGaugh, 1966; 1989; McGaugh and Petrinovich, 1963). In support of this, it was reported that post-training strychnine (McGaugh, Thomson, Westbrook, and Hudspeth, 1962) and several other compounds that have similar central nervous system actions (Breen and McGaugh, 1961; McGaugh, Westbrook, and Thomson, 1962) injected up to 15 minutes after training facilitated maze learning. Injections given 30 or 90 minutes after training had no effect. Posttraining strychnine and picrotoxin were also found to improve retention of an avoidance task (Bovet, McGaugh, and Oliverio, 1966). Posttraining injection of a strychnine-like compound (1757 I.S.) was also found to facilitate latent learning (as measured by enhanced performance during the rewarded phase) when administered each day after the non-rewarded phase (Westbrook and McGaugh, 1964).

The authors (Bovet, McGaugh, and Oliverio, 1966; Breen and McGaugh, 1961; McGaugh, Thomson, Westbrook, and Hudspeth, 1962; McGaugh, Westbrook, and Thomson, 1962; Westbrook and McGaugh, 1964) suggested that posttraining administration of the compounds enhanced the reverberating neural activity enhancing the memory trace produced by the training. Enhanced reverberating activity may have promoted the synaptic changes thought to mediate more permanent memory representations (Hebb, 1955; McGaugh and Herz, 1972; Milner, 1957). Such effects on the consolidation process are called memory modulation (Cahill and McGaugh, 1996; Gold and McGaugh, 1975; McGaugh, 1966; 2000;

McGaugh and Petrinovich, 1963).

A number of other experimental treatments have been found to modulate memories of a task if they are administered during a limited period of time after training (for reviews see Cahill and McGaugh, 1996; Glickman, 1961; McGaugh and Cahill, 1997; McGaugh, Cahill, Ferry, and Roozendaal, 2000; White, 1998). Posttraining treatments administered after the consolidation phase do not modulate memory (Gerard, 1961; McGaugh, 1966; 1989; 2000) and are used as controls to determine if a particular treatment genuinely modulates the time-dependent memory processes.

Compared to saline injected controls, rats injected subcutaneously with epinephrine immediately after passive avoidance training (Gold and van Buskirk, 1975) significantly increased their latency to enter the shock compartment 24 hours later. Immediate posttraining amphetamine injections have been found to improve the retention of a shuttle-box active avoidance task (Evangelista and Izquierdo, 1971), a passive avoidance task (Johnson and Waite, 1971), and the retention of tone-shock pairings measured as elevated suppression during presentation of the tone (Carr and White, 1984). Furthermore, posttraining ingestion or injection of glucose (Messier and White, 1984; 1987) has been shown to improve the retention of tone-shock pairings as measured by an increase in the suppression ratio during presentation of the tone. Other demonstrations of memory improving effects produced by posttraining glucose are reported by Gold and colleagues (Gold, Vogt, and Hall, 1986; for reviews see Gold, 1992; 1995).

A number of studies have reported that posttraining aversive events can also modulate the retention of recently acquired information. Rats given foot shock immediately but not 30 minutes after tone-shock pairings (White and Legree, 1984) displayed elevated suppression of drinking during presentation of the tone 24 hours later compared to rats that received similar training but had not been shocked after training. In another study, foot shock administered immediately but not 2 hours after passive avoidance training (Jodar, Takahashi, and Kaneto, 1996) significantly prolonged step-through latency compared to rats that did not receive posttraining foot shocks. A stressful swim has also been shown to improve performance of a passive avoidance task (Flint, Metzger, Benson, and Riccio, 1997) when the

swim occurred immediately following training but not 15 minutes posttraining. Immediate shock has also been shown to modulate the retention of an appetitive Y-maze discrimination task (Holahan and White, 2002).

Memory modulation also results from posttraining exposure to an aversive CS. In a demonstration of conditioned memory modulation, groups of rats were given tone-shock pairings in one shuttle-box compartment (CS) and no shocks in the adjacent compartment (Holahan and White, 2002). The same rats were then trained on a Y-maze to enter one arm for food and avoid a second arm that contained no food and exposed to the shock or no shock compartment immediately after the final training trial. When tested 24 hours later on the Y-maze, rats exposed to the aversive CS in the shock compartment immediately but not 2 hours after training made more correct arm entries than rats exposed to the no shock compartment. Thus, posttraining exposure to unconditioned and conditioned aversive stimuli modulate memory in a similar fashion.

2. Memory modulation and the amygdala

a. Unconditioned Memory Modulation

Early experiments using electrical stimulation of the amygdala indicated that it is involved in memory consolidation. Stimulation in the vicinity of the BLA (Goddard, 1964) following tone-shock pairings disrupted subsequent suppression of behavior (pressing for food pellets) in the presence of the tone CS. Posttraining stimulation of the BLA has also been found to produce both short- and long-term disruption of an aversive task (Kesner and Conner, 1974) but stimulation with the same parameters (Berman and Kesner, 1976) following training on an appetitive task did not disrupt retention. Immediate posttraining amygdala stimulation (no specific region mentioned) after passive avoidance training disrupted performance when rats were trained with a 2 mA foot shock but enhanced performance when rats were trained with a 0.5 mA foot shock (Gold, Hankins, Edwards, and Chester, 1975).

Studies using lesion techniques or intra-amygdala injections of various pharmacological agents have also found that the amygdala is involved in memory consolidation. In one study, amygdala lesions were made immediately or 2, 5 or 10 days after

inhibitory avoidance training (Liang *et al.*, 1982). Since lesions within 2 days of training impaired retention and lesions at 10 days had no effect, the authors argued that the amygdala was critical during a limited posttraining period. Additional studies found that intra-amygdala injections of a β-adrenergic antagonist (Gallagher, Kapp, Musty, and Driscoll, 1977), an opiate agonist (Gallagher and Kapp, 1978), lidocaine (Parent and McGaugh, 1994), muscimol (Wilensky, Schafe, and LeDoux, 2000) or tetrodotoxin (Bucherelli, Tassoni, and Bures, 1992) immediately after passive avoidance training impaired retention when rats were tested 24 hours later. Posttraining injections into the BLA but not CeA of the corticotrophin releasing hormone (CRH) antagonist α-helical CRH (0.3, 1.0, or 3.0 μg) blocked retention of an inhibitory avoidance task (Roozendaal, Brunson, Holloway, McGaugh, and Baram, 2002). Posttraining injections of lidocaine (Vazdarjanova and McGaugh, 1999), an extracellular signal-regulated kinase/ mitogen-activated protein kinase (ERK/MAPK) inhibitor (Schafe *et al.*, 2000) or a protein synthesis inhibitor (Schafe and LeDoux, 2000) in the LA/BLA impaired the retention of aversive Pavlovian conditioning as measured by freezing.

Disruption of normal amygdala activity or its outputs (e.g., the stria terminalis; for anatomy see Pitkänen, 2000; Sarter and Markowitsch, 1985; for behavioral data see Liang and McGaugh, 1983a; 1983b; Liang, McGaugh, and Yao, 1990; McGaugh, Introini-Collison, Juler, and Izquierdo, 1986) have been shown to block the memory modulating effects of a variety of systemically and centrally administered unconditioned stimuli (for review see McGaugh, 2002; McGaugh, Cahill, Ferry, and Roozendaal, 2000; McGaugh, Cahill, and Roozendaal, 1996).

An early report (Van Wimersma Greidanus, Croiset, Bakker, and Bouman, 1979) found that lesions of the CeA/BLA blocked the modulating effects of posttraining vasopressin or ACTH injections on enhanced extinction of an avoidance task. Large posttraining lesions of the entire amygdala complex blocked the memory modulating effect of posttraining epinephrine injections on the retention of inhibitory avoidance (Cahill and McGaugh, 1991). As these lesions also blocked unmodulated retention of the inhibitory avoidance behavior, it is difficult to attribute the effect of the lesion to a disruption of memory modulation. Electrolytic lesions of the CeA/BLA have also been found to block the memory-enhancing

effect of posttraining bicuculline injections and the memory-impairing effect of posttraining muscimol injections on the retention of inhibitory avoidance (Ammassari-Teule, Pavone, Castellano, and McGaugh, 1991). Posttraining dexamethosone injections modulated the retention of inhibitory avoidance in sham lesioned animals and animals with ibotenic acid lesions of the CeA (Roozendaal and McGaugh, 1996). The modulation produced by dexamethosone was not observed in animals with either ibotenic acid lesions of the MeA or NMDA lesions of the BLA.

Specific pharmacological manipulations of the amygdala have provided evidence for possible neurochemical mechanisms underlying amygdala-mediated memory modulation. The enhancement of inhibitory avoidance produced by posttraining dexamethasone injections (0.3 or 1.0 mg/ kg) was blocked by injections of selective β1 or β2 adrenergic antagonists into the BLA but not CeA (Quirarte, Roozendaal, and McGaugh, 1997) and by intra-BLA atropine (0.5 μg/ 0.2 μl) injections (Power, Roozendaal, and McGaugh, 2000). Intra-CeA atropine (1.0 μg/ 0.5 μl) injections also blocked the enhanced retention for inhibitory avoidance produced by posttraining systemic injections of the muscarinic agonist oxotremorine (Introini-Collison, Dalmaz, and McGaugh, 1996). These studies indicate that intrinsic amygdala processes mediate unconditioned memory modulation.

The hypothesis that intrinsic amygdala processes mediate memory modulation is supported by findings based on injections of substances directly into the amygdala as the posttraining treatment. Immediate posttraining injections of corticotropin releasing factor into the LA at a medium dose (0.1 μg) significantly improved retention of passive avoidance (Liang and Lee, 1988) compared to posttraining injections of a low (0.01 μg) or high (1.0 μg) dose. Immediate posttraining injections of a benzodiazepine antagonist (flumazenil, 10 nmole) into the CeA/LA (Izquierdo, Da Cunha, Huang, and Walz, 1990) facilitated the retention of step-down passive avoidance training. Posttraining infusions of a glucocorticoid receptor agonist into the BLA but not CeA (Power, Roozendaal, and McGaugh, 2000; Roozendaal and McGaugh, 1997a) immediately after one-trial passive avoidance training enhanced retention. Likewise, posttraining injections of norepinephrine into the BLA enhanced retention of a spatial water maze task (Hatfield and McGaugh, 1999).

Evidence has also shown that the amygdala modulates information stored in other brain regions. Immediate posttraining injections of amphetamine into the hippocampus enhanced memory for hidden platform and injections into the dorsal striatum enhanced memory for visible platform water maze tasks (Packard, Cahill, and McGaugh, 1994; Packard and Teather, 1998) while infusions into the BLA enhanced retention of both tasks. Lidocaine injected into the BLA before testing did not disrupt retention (Packard and Teather, 1998), suggesting that the amygdala modulated the memories required to perform the tasks but did not store these memories.

An intact amygdala may also be required for enhanced storage of memories located elsewhere in the brain. NMDA lesions of the BLA but not ibotenic acid lesions of the CeA blocked the enhancing effect of a glucocorticoid agonist injected into the hippocampus after inhibitory avoidance training (Roozendaal and McGaugh, 1997b). Posttraining injections of glutamate into the hippocampus enhanced memory for a win-shift task, which was blocked by concurrent injections of lidocaine into the BLA (Packard and Chen, 1999). Injections of a β-adrenoceptor antagonist into the BLA blocked the enhanced modulation of memory for inhibitory avoidance training produced by ipsilateral injections of a glucocorticoid agonist into the hippocampus (Roozendaal, Nguyen, Power, and McGaugh, 1999). Unilateral injections of 8 Br-cAMP (0.25 μg or 1.25 μg) into the entorhinal cortex enhanced the retention of inhibitory avoidance (Roesler, Roozendaal, and McGaugh, 2002). This enhancement was blocked in rats with ipsilateral but not contralateral NMDA lesions of the BLA.

b. Conditioned memory modulation

Pretraining electrolytic lesions of the CeA or MeA but not the BLA or LA blocked the enhanced retention of an appetitive Y-maze task produced by posttraining exposure to an aversive CS (Holahan and White, 2002). However, it is unclear from this study whether the lesions were specific to the modulation produced by the CS or if they affected performance on the Y-maze task used to measure the conditioned memory modulation. This issue is examined in the present thesis.

3. Summary of modulation studies

A variety of posttraining amygdala manipulations can enhance or disrupt the retention

of recently acquired memories. Lesions or pharmacological manipulations of the amygdala block the ability of systemically administered unconditioned or conditioned treatments to modulate recently acquired memories. These effects appear to based on intrinsic amygdala processes. The amygdala also appears to interact with other brain structures in the modulation and storage of memories. These findings have raised a major question concerning amygdala involvement in memory processes. Does memory storage take place within the amygdala or does the amygdala serve only to modulate memories stored in other brain regions? A general theme of the present thesis examines this debate.

E. Neural activation

One way to study the function of particular brain regions during aversive conditioning is to determine what types of environmental or behavioral manipulations activate them. One measure of neural activity involves immunohistochemical labeling of immediate early gene or protein induction.

1. C-Fos protein expression

Once a threshold is surpassed (Shin, McNamara, Morgan, and Curran, 1990), neural activation produces a variety of cellular events that result in long-term changes in the structure and function of the cell (for reviews see Malenka and Nicoll, 1999; Sanes and Lichtman, 1999). The induction of immediate early genes and proteins are among these events (Goelet, Castellucci, Schacher, and Kandel, 1986; Morgan and Curran, 1991; Sheng and Greenberg, 1990). Immediate early genes such as *c-fos*, *jun*, *zif268*, *Arc*, or *Homer 1* and their protein products are transiently induced by a variety of hormones, drugs, and other unconditioned and conditioned stimuli (Clayton, 2000).

It has been suggested that immediate early gene protein products have two broad functions in the cell. When induced, direct effector proteins have structural or enzymatic roles that have an immediate impact on the cellular structure or function (Clayton, 2000). The direct effector proteins may facilitate the stabilization of recent changes in synaptic efficacy (Guzowski, 2002; Malenka and Nicoll, 1999; Sanes and Lichtman, 1999). Induction of these direct effector proteins may directly result in neuronal sprouting, increased synaptic density, production of cytoskeletal proteins, or expression of ion channels and receptors

(Lanahan and Worley, 1998; Sheng and Greenberg, 1990). Examples of direct effector proteins include ARC (Clayton, 2000; Lanahan and Worley, 1998) and GAP-43 (Benowitz and Routtenberg, 1997) which contribute to the production of cytoskeletal proteins and HOMER, which augments receptor function (Clayton, 2000; Lanahan and Worley, 1998).

Immediate early gene proteins also regulate the transcription of additional genes directing a cell's genomic response to a variety of environmental stimuli (Sheng, McFadden, and Greenberg, 1990). These regulatory proteins, such as c-Fos, control downstream gene expression and are thought to translate environmental signals into relatively long-term changes in neuronal function (Goelet, Castellucci, Schacher, and Kandel, 1986; Kaczmarek and Chaudhuri, 1997; Sheng, McFadden, and Greenberg, 1990). It has been suggested that when these regulatory proteins are translated they return to the nucleus to regulate the expression of late response genes whose products are thought to directly subserve the encoding of structural and functional changes responsible for long-term synaptic alteration (Carew, 1996). If this view is correct, it would mean that expression of the c-Fos protein may provide a marker for cells that have recently been activated and are potentially undergoing long-term structural or functional changes.

In the present thesis, expression of the c-Fos protein was used as a marker for recently activated cells. The function of the c-Fos protein depends on its binding with Jun proteins (Sheng and Greenberg, 1990). These Fos/Jun protein complexes stimulate transcription (c-Fos/c-Jun dimer) or repress transcription (Fos/JunB dimer) (Sheng and Greenberg, 1990). Therefore, the c-Fos protein may activate or repress intracellular direct effector proteins leading to enhanced or depressed cellular function (Kaczmarek, 1993; Kaczmarek and Nikolaev, 1990). In the present thesis, the c-Fos protein was used as a measure of cellular activity indicative of potential long term changes in the cells that express it.

2. Amygdala and c-Fos protein expression

Using c-Fos mRNA or protein immunohistochemistry, investigators have analyzed the relationship between unconditioned or conditioned stimulation and activity changes in the amygdala. These studies are most often carried out to determine the response of amygdala neurons to exposure to aversive stimuli. However, since exposure to aversive stimuli elicits

behavioral output, one issue to consider is the distinction between activation of amygdala neurons produced by behavioral output and that produced by environmental stimuli. A related issue is whether amygdala activation produces behavioral output. These issues have not been extensively covered in the literature.

a. Unconditioned activation

A variety of unconditioned aversive stimuli induce the c-Fos protein or gene in the amygdala. Electrical or chemical stimulation of the periacqueductal gray or medial hypothalamus produced unconditioned defensive behaviors and induced high levels of amygdala *c-fos* mRNA expression (Sander, *et al.*, 1993). Handling rats for the first time also elevated amygdala *c-fos* mRNA levels with a subsequent decline after repeated handling (Campeau, *et al.*, 1991). Exposure to a loud tone, which produced a variety of unconditioned defensive behaviors, elevated c-Fos protein expression in both the BLA and CeA (Beckett, Duxon, Aspley, and Marsden, 1997). Exploration of a novel context elevated *c-fos* mRNA induction in the BLA and MeA compared to a home cage control group (Hess, Gall, Granger, and Lynch, 1997) with a subsequent decline after repeated exploration.

Unconditioned foot shock also activates the amygdala as measured with c-Fos gene and protein products. Campeau, *et al.* (1991) reported that foot shock elevated amygdala complex *c-fos* mRNA levels. This elevated mRNA labeling was localized in the MeA following shock administration (Pezzone, Lee, Hoffman, and Rabin, 1992; Rosen, Fanselow, Young, Sitcoske, and Maren, 1998). In addition to finding elevated *c-fos* in the MeA, Rosen, *et al.*, (1998) also reported that another immediate early gene, *NGFI-A* (a.k.a. *zif/268*), was induced in the LA following shock. This suggests that cells expressing proteins other than c-Fos may also be activated by aversive stimuli.

Using a delayed shock procedure, CeA c-Fos labeling was elevated following footshock (Milanovic, *et al.*, 1998) in both immediate and delayed foot shock groups. Only the delayed foot shock group showed evidence of learning suggesting that the shock itself rather than an associative process produced the elevated labeling. In a similar demonstration (Radulovic, Kammermeier, and Spiess, 1998) CeA c-Fos levels were elevated following shock both in mice that were pre-exposed and in mice that were not pre-exposed to the shock

context. Only the pre-exposed mice exhibited learning 24 hours later, leading to a similar conclusion that shock elevated amygdala c-Fos expression independently of an associative process. It has also been reported (Savonenko, Filipkowski, Werka, Zielinski, and Kaczmarek, 1999) that c-Fos protein expression was elevated in the LA, BLA, and MeA but not the CeA following one session of two-way active avoidance training using shock. No significant correlation was found between c-Fos levels and the number of avoidance behaviors. These results suggest that amygdala activity, as measured with c-Fos expression, results from a variety of unconditioned aversive stimuli independently of the formation or expression of conditioned associations.

b. Conditioned activation

Exposure to conditioned aversive cues has also been found to elevate c-Fos protein and mRNA expression in the amygdala. Campeau, *et al.*, (1991) reported that confining rats in a compartment where shock had previously been given elevated amygdala complex *c-fos* mRNA. A similar procedure (Beck and Fibiger, 1995) elevated levels of "crouching" (i.e., freezing) and c-Fos protein expression in the CeA, BLA, and MeA.

Other studies have examined amygdala c-Fos expression to an aversive CS by manipulating the conditioning process. In one study (Milanovic, Radulovic, Laban, Stiedl, Henn, and Spiess, 1998), c-Fos protein expression was assessed during re-exposure to a shock-conditioned context in mice that were trained with immediate foot shock, delayed foot shock (180 seconds after placement into the compartment), or no shock. Re-exposure to the shock-conditioned context elevated freezing and CeA c-Fos expression in the delayed foot shock but not in the immediate foot shock group. The effects in the delayed foot shock group may have been due to a conditioning process. Alternatively, freezing could have resulted directly from the neural activation implied by the elevated c-Fos expression, or elevated c-Fos expression could have been caused by feedback from the expression of freezing.

In a second study (Hall, Thomas, and Everitt, 2001) the contingency between a shock US and an auditory CS was manipulated by presenting either paired or random presentations of a clicker and shock. When tested in the absence of shock, levels of freezing were higher following presentation of the shock-paired clicker than the unpaired clicker. Induction of c-

Fos protein labeling in the BLA was higher in the group exposed to the paired clicker than in the group exposed to the unpaired clicker and a home cage control. Labeling in the CeA was higher in the paired group than the home cage control group. There was no effect in LA.

A third study decreased the associability of the CS by giving rats nonreinforced presentations of the CS prior to training (latent inhibition design; Chacto and Lubow, 1967; Lubow, 1965; Lubow and Moore, 1959). In this study (Sotty, Sander, and Gosselin, 1996), rats given nonreinforced presentations of the light CS showed low levels of conditioning and low levels of amygdala *c-fos* mRNA labeling. Rats conditioned without nonreinforced CS presentations exhibited more conditioning and showed elevated *c-fos* mRNA expression in the BLA and CeA. The elevated c-Fos expression and conditioned suppression in the conditioned group could have been based on an associative process or been due to feedback from the suppression of behavioral output. It is also possible that elevated neural activity in the conditioned group produced the conditioned suppression.

3. Summary of c-Fos studies

The results of the c-Fos investigations cited above indicate that the c-Fos protein is induced in amygdala subregions following presentation of unconditioned or conditioned aversive stimuli. However, it is not clear whether the elevated c-Fos expression is based on a conditioning process, the sensory properties of the US and CS, or feedback from the behavioral output. Furthermore, it is not clear whether amygdala activation produces freezing directly or indirectly. These issues are addressed in the present thesis.

F. Anatomy

Based on its efferent and afferent connections (for review see Pitkänen, 2000), the amygdala may be a pivotal structure that receives sensory input and mediates information that alters the production of internal responses and overt behaviors. As shown in Figure 1, the amygdala complex can be segregated into 4 main subnuclei. Figure 1 also shows that these subnuclei receive inputs from a wide variety of sensory regions and project to multiple brain regions involved in motor and autonomic control (reproduced based on a drawing from Pitkänen, 2000 Fig 2.26, p. 98).

1. Lateral Amygdala (LA)

The lateral amygdala (LA) includes the dorso-lateral, ventro-medial, and ventro-lateral subdivisions. This nucleus receives cortical afferent projections from auditory and somatosensory association areas (LeDoux, Cicchetti, Xagoraris, and Romanski, 1990; Romanski and LeDoux, 1992). The LA also receives sensory inputs from the acoustic thalamus (LeDoux, Cicchetti, Xagoraris, and Romanski, 1990; Romanski and LeDoux, 1992). In addition to these auditory inputs, the LA receives substantial input from association cortices such as the caudal orbital cortex (van Hoesen, 1981) and the perirhinal and parahippocampal cortices (Turner, Mishkin, and Knapp, 1980; van Hoesen, 1981). The insula cortex has also been shown to project to LA (Friedman, Murray, O'Neil, and Mishkin, 1986). These anatomical data indicate that the LA may be a key site for sensory information to enter the amygdala complex.

2. Basolateral Amygdala (BLA)

The basolateral amygdala (BLA) consists of anterior, posterior, and ventral subdivisions. The perirhinal and parahippocampal cortices (Turner, Mishkin, and Knapp, 1980; van Hoesen, 1981) as well as prefrontal cortex and cingulated gyrus send strong projections to the BLA (van Hoesen, 1981). Additionally, insular cortex, medial and lateral orbital cortices, and the medial region of the frontal lobe (Pitkänen, 2000) project to BLA. There are also reciprocal connections from BLA to these cortical regions (Pitkänen, 2000).

The BLA projects back to polysensory cortices (Morán, Mufson, and Mesulam, 1987) such as agranular insula, the medial orbital cortex, and the ventromedial portion of the prefrontal cortex (Barbas and de Olmos, 1990). The BLA also projects to perirhinal cortex and hippocampus (Pitkänen, 2000). Cells in the BLA form overlapping clusters that project topographically to frontal, insular, and cingulate cortices (Barbas and de Olmos, 1990). The medial portion of the orbital cortex receives a projection from the medial parts of the BLA and the lateral orbital area receives input from the lateral portion of the BLA (Barbas and de Olmos, 1990). These projections could allow the BLA to modulate cortical information processing during aversive conditioning (McGaugh, 2000; Paré, Collins, and Pelletier, 2002).

One of the main features that distinguishes the BLA from other amygdala subnuclei is its strong projections to the dorsal and ventral striatum (Kelley, Domesick, and Nauta,

1982; McDonald, 1991a) and its vast intra-amygdaloid projections to the medial (MeA) and central (CeA) amygdala nuclei (McDonald, 1991a; 1991b). The amygdala projection to the striatum arises exclusively from the BLA (Parent, Mackey, and DeBellefeuille, 1983; Pitkänen, 2000) with no projections to the striatum originating in CeA or LA (Parent, Mackey, and DeBellefeuille, 1983). The BLA-striatum projections terminate preferentially in the nucleus accumbens and ventral parts of the caudate-putamen (ventral and medial parts that border the nucleus accumbens; (Amaral, Price, Pitkänen, and Carmichael, 1992; Kelley, Domesick, and Nauta, 1982). This could be a route whereby the amygdala influences motor output (Burns, Annett, Kelley, Everitt, and Robbins, 1996; Burns, Robbins, and Everitt, 1993).

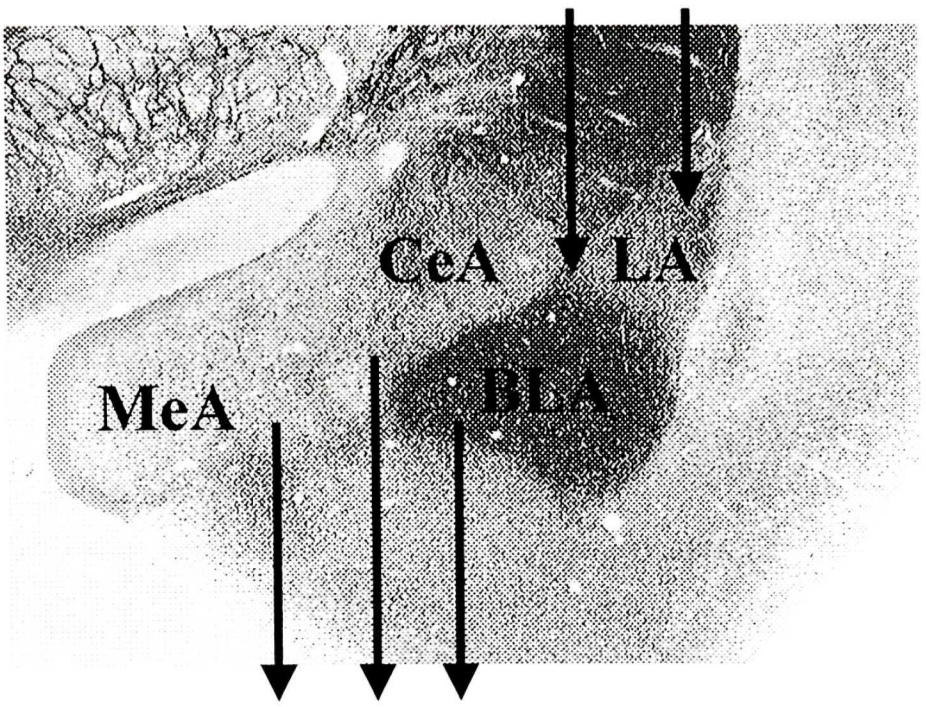
3. Central Amygdala (CeA)

The central amygdala (CeA) consists of medial, lateral, and central subdivisions. The CeA sends strong efferent projections to and receives afferent projections from various brainstem areas such as the periacqueductal gray, parabrachial nuclei, and the caudal medulla (de Olmos, Alheid, and Beltramino, 1985). The CeA also receives a direct projection from the spinal cord (Burstein and Potrebic, 1993). There is also evidence that the rostral insula and orbital cortex project directly to CeA (van Hoesen, 1981) suggesting the possibility for direct somatosensory input to CeA.

Figure 1. Acetylcholinesterase stained section from a rat brain corresponding to -2.80 mm from bregma (Paxinos and Watson, 1998). The amygdala shown is from the left hemisphere. Abbreviations are LA: lateral amygdala, BLA basolateral amygdala, CeA central amygdala, MeA medial amygdala. Inputs from various cortical and subcortical areas terminate preferentially in the LA, BLA, and MeA. Outputs to cortical and subcortical areas originate preferentially in the BLA, CeA and MeA.

Sensory Input

Cortex
Thalamus
Midbrain
Hypothalamus



Behavioral and Autonomic Output

Cortex
Striatum
Hypothalamus
BNST
Thalamus
Hippocampus
Brainstem

The bed nucleus of the stria terminalis provides a relay for projections from CeA to the brain stem and hypothalamus (Holstege, Meiners, and Tan, 1985). In the rat, the lateral subdivision of the CeA projects to the lateral part of the bed nucleus whereas the medial and posterior subdivisions project to the medial part of the bed nucleus (Krettek and Price, 1978a; Krettek and Price, 1978b). The CeA projects to the ventrolateral and dorsomedial portions of the periacqueductal gray, the solitary tract and dorsal motor nucleus of the vagus, and the parabrachial nucleus (which returns a projection to CeA; Price and Amaral, 1981). It has been argued that projections from CeA to brain stem play an important role in the expression of freezing (see Carrive, Lee, and Su, 2000; Carrive, Leung, Harris, and Paxinos, 1997; Carrive, 1993; Fendt and Fanselow, 1999).

Based on the anatomical connections, the amygdala may be in a position to influence the expression of motor behaviors through two pathways; one from BLA to striatum as this pathway has been shown to be involved in increases in locomotor activity (Burns, Annett, Kelley, Everitt, and Robbins, 1996; Burns, Robbins, and Everitt, 1993) and another from CeA to periacqueductal gray as this pathway has been shown to be involved in the suppression of motor behaviors (Carrive, Lee, and Su, 2000; LeDoux, Iwata, Cicchetti, and Reiss, 1988).

4. Medial Amygdala (MeA)

The medial amygdala (MeA) includes posterior dorsal, anterior dorsal, posterior ventral, and anterior cortical subdivisions. The MeA has strong efferent projections to and afferent projections from the hypothalamus, the horizontal limb of the diagonal band, and the septum (de Olmos, Alheid, and Beltramino, 1985). Projections originating in the MeA terminate in the anterior hypothalamus as well as the "core" of the ventromedial hypothalamus (Krettek and Price, 1978a). Projections also terminate in the lateral hypothalamus (Krettek and Price, 1978a). These connections may provide a route for the amygdala to influence changes in internal responses.

V. The Present Thesis

Four measures of the effects of aversive stimuli have been described: freezing, avoidance, memory modulation, and c-Fos protein expression. Studies have been reviewed showing that c-Fos activation and memory modulation occur following exposure to aversive

USs and CSs while observations of freezing and place avoidance occur during exposure to aversive CSs. Several issues remain to be examined to gain a better understanding of the relationship among these measures and the role of the amygdala in producing them.

Although freezing itself appears to be an unlearned behavior (Bolles, 1970), the range of stimuli that elicit it is partly determined by experience. Therefore, the first question pertains to whether or not manipulations of the amygdala block the innate ability to produce freezing or whether they impair a learning process, the results of which influence the occurrence of freezing. It may also be the case that impaired amygdala function disrupts both the innate ability to produce the behavior and the learned association.

To differentiate between these hypotheses, the experiments described in Manuscript 1 examined both freezing and activation of amygdala neurons (inferred from c-Fos protein immunohistochemistry) following exposure to a shock US and a shock-paired contextual CS. Comparing amygdala activity and freezing provided an indication of amygdala involvement in various conditioning stages and the relationship of amygdala activity to freezing.

A second issue is the incompatibility of freezing and active place avoidance. Freezing in the presence of aversive stimuli may interfere with the ability to actively avoid those stimuli. Freezing and active avoidance are therefore incompatible because one can not occur in the presence of the other (Anisman, 1973; Anisman and Waller, 1972; 1973). As reviewed, amygdala manipulations reliably eliminate freezing, while similar manipulations have not reliably eliminated active avoidance. Since these behaviors (freezing and active avoidance) are in competition with each other, they provide a way in which to further study whether amygdala manipulations result in performance deficits or associative deficits.

A related issue concerns the testing apparatus used to examine freezing or active avoidance. In the standard test for freezing, there is no way for an animal to actively avoid the CS (no route for escape or available response that terminates the aversive CS). In an avoidance test, some behavior ultimately leads to decreased exposure to the aversive CS. In the standard test for freezing, absence of freezing leads to the conclusion that the aversive association has been disrupted. However, the rat may still show avoidance of the aversive CS if tested appropriately (cf., Darwin, 1972).

In Manuscript 2, the argument over whether the amygdala is required for the production of specific overt behaviors was examined by injecting muscimol into the amygdala before context-shock pairings or re-exposure to the CS and measuring freezing and place avoidance. If amygdala inactivation blocked the expression of both behaviors, it would be difficult to interpret these results in terms of performance deficits. Rather, the findings would suggest that the amygdala may be part of a neural system that mediates mnemonic representations that indirectly promote overt behaviors.

A final issue concerns the use of conditioned memory modulation to study associative mechanisms in aversive conditioning. Studying the memory modulating effects of a CS can provide a method for inferring the existence of an array of internal responses that can be measured independently of its immediate behavioral effects. This is because the modulating effects of the CS are observed at a later time as an effect on the retention of previously acquired information (Cahill and McGaugh, 1996; McGaugh, 1966; 2000).

Measurement of memory modulation in the present thesis following exposure to an aversive CS is taken as an additional observation that requires the inference of internal responses produced by exposure to aversive stimuli, and an indication of whether amygdala manipulations produce behavioral or associative deficits. Manuscript 3 reports on the measurement of memory modulation in subjects exposed to an aversive CS during the posttraining period. During the posttraining phase, freezing and place avoidance were also measured. If inactivation of the amygdala blocked the production of internal responses, memory modulation, freezing, and place avoidance should be eliminated. If amygdala inactivation produced specific deficits in the expression of overt behaviors, either freezing or place avoidance (or both) would be reduced but not memory modulation.

Manuscript 1

The hypothesis that amygdala lesions impair the ability to freeze implies that the occurrence of freezing and the activity of some population of amygdala neurons should be closely related. Identification of a population of neurons that is activated in the absence of freezing or not activated during the occurrence of freezing would indicate that their function is not directly related to producing the behavior. In this case the activity might be due to exposure to the US, the CS, or both. Such activity could also reflect a mnemonic process that allows the CS to produce freezing. In Manuscript 1, neural activity in the amygdala was inferred from the induction of the immediate early gene protein product, c-Fos. This was done to examine the relationship between freezing and amygdala c-Fos protein expression following exposure to unconditioned (Experiment 1) and conditioned (Experiment 2) aversive stimuli. The occurrence of freezing was manipulated to test the hypothesis that the activity of a population of neurons revealed by c-Fos activation would track the changes in freezing produced by these manipulations.

Amygdala c-Fos induction corresponds to unconditioned and conditioned aversive stimuli but not to freezing.

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Abstract

Freezing and amygdala c-Fos protein labeling were compared following exposure to footshock or to shock-conditioned contextual cues. In Experiment 1 freezing was elevated during a period immediately following shock in rats that remained in the shock context, but not in rats that were moved to a different, neutral context. Both of these groups showed equally elevated c-Fos levels in the central (CeA) and lateral (LA) nuclei. In Experiment 2 rats were shocked in one compartment (paired) and not shocked in another, distinct compartment (unpaired). Rats re-exposed to the paired compartment 24 hr later froze more than rats exposed to the unpaired compartment. Rats in both of these groups froze more than non-shocked rats. c-Fos protein expression in CeA, LA and basolateral nucleus (BLA) was elevated in the rats exposed to the paired compartment compared to rats exposed to the unpaired compartment and to the non-shocked controls, which had similar levels of c-Fos induction. c-Fos expression was induced by exposure to both unconditioned and conditioned stimuli, although it is unclear if the same cell population was activated in both cases. Neither case of c-Fos expression was correlated with the occurrence of freezing. c-Fos expression may represent neural activity in LA and CeA produced by exposure to unconditioned cues and activity in BLA, LA and CeA produced by conditioned cues. This activity may contribute to an aversive affective state (or "fear"). Behaviors promoted by this state, such as freezing, may be mediated in other brain areas.

Theme: Neural basis of behavior

Topic: Learning and memory: systems and functions - animals

Key Words: amygdala, conditioned fear, freezing, c-Fos, context conditioning

Introduction

The amygdala has been implicated in both appetitive and aversive conditioning [for reviews see 22,24,34,52] although the precise nature of its involvement is controversial [e.g., 12,30]. In one procedure used to study the function of the amygdala, footshock is paired with a cue (e.g., a temporally discrete tone or light) in a specific context. Behavioral changes observed during subsequent exposure to the cue and the context (either separately or together) suggest that both have become aversive conditioned stimuli (CS). Evidence collected using these Pavlovian conditioning procedures shows that the amygdala participates in the acquisition, storage, modulation, and retrieval of information that contributes to the altered behaviors [for reviews see 22,32,52,58,62; see also 44,95,98].

Freezing is a behavior commonly observed during exposure to an aversive CS [5,7,8,25,53,61] and its occurrence is sensitive to amygdala manipulations. Freezing during exposure to a compartment previously paired with shock is attenuated by pre-training electrolytic lesions of the amygdala complex [75], radiofrequency lesions of the medial (MeA) amygdala [6], electrolytic lesions of the central (CeA) or basolateral (BLA) nuclei [44,51] and by neurotoxic lesions centered on the BLA [56,57,94], the CeA or the lateral nucleus (LA) [36]. Muscimol or NMDA antagonists disrupt freezing when injected into the BLA immediately before training [28,41,71] or testing [31,41,55,59,71], but not when injected immediately post-training [97].

Two general hypotheses have been proposed to explain the freezing deficits produced by amygdala manipulations. It is possible that the amygdala (or certain parts of it) is a critical structure for the conditioning process, and that impairing its function compromises a circuit that stores information required for aversive CSs to produce behavioral changes such as increases in freezing [30,32,52,57,58]. Alternatively, it has been argued that reductions in freezing produced by lesions or inactivation of the amygdala may result from impairments in the rats' ability to produce the behavior [11,12,93]. The present study examined this issue by comparing a measure of amygdala neural activity produced by exposure to a US and a CS with the occurrence of freezing produced by the same stimuli.

The hypothesis that amygdala lesions impair the ability to freeze implies that the

occurrence of freezing and the activity of some population of amygdala neurons should be closely related. Identification of a population of neurons that is activated in the absence of freezing or not activated during the occurrence of freezing would indicate that their function is not directly related to producing the behavior. In this case the activity might be due to exposure to the US, the CS, or both. Such activity could also reflect a mnemonic process that allows the CS to produce freezing.

In the present study, neural activity in the amygdala was inferred from the induction of the immediate early gene protein product, c-Fos. Genes such as *c-fos*, *c-jun* and *zif/268* and their respective protein products are induced by a variety of stimuli [42] and have been used as indicators of neural activation [68,83]. Since the c-Fos protein regulates the transcription of other genes involved in synaptic changes hypothesized to underlie learning and memory [1,15,18,33,35,79,80,86] it has also been suggested that this protein may be a marker for recent neuroplastic events in the neurons that express it [18,38,45-47,92].

Using c-Fos mRNA or protein immunocytochemistry, investigators have analyzed the effects of exposure to USs and CSs in various brain regions [cf., 13,23,72,78,85,87] including the amygdala. Unconditioned foot shock increases *c-fos* mRNA levels in the whole amygdala [14] and specifically in the MeA [74,81] and CeA [66]. In the LA, Rosen et al., [81] found elevated expression of another immediate early gene, *NGFI-A* (a.k.a. *zif/268*), but not of *c-fos*.

Exposure to a shock-conditioned context elevated c-Fos protein expression in the whole amygdala [14] and specifically in the CeA and BLA [3,66]. Exposure to a shock-paired but not an unpaired auditory CS elevated c-Fos protein expression in the BLA and CeA, but not in LA [40]. Latent inhibition of a shock-paired light CS reduced both suppression of drinking and c-Fos protein expression in the BLA and CeA [88]. Using a delayed- shock training procedure, mice tested in the conditioned context 24 hours later froze more and had elevated c-Fos expression in the BLA and CeA compared to mice that had been shocked immediately after being placed into the context [66,89].

The purpose of the present study was to examine the relationship between freezing and amygdala c-Fos protein expression following exposure to unconditioned (Experiment 1)

and conditioned (Experiment 2) aversive stimuli. In both experiments the occurrence of freezing was manipulated to test the hypothesis that the activity of a population of neurons revealed by c-Fos activation would track the changes in freezing produced by these manipulations.

2. Experiment 1

In Experiment 1 rats were shocked and either remained in the presence of the aversive contextual CS or were immediately switched to a neutral context. As reported previously [7,8,25] switching rats in this way reduces freezing in the neutral context. This has been interpreted to suggest that immediate post-shock freezing is elicited by conditioned stimulus properties of the shock-paired context rather than by the US [25-27,29,50,82]. Following this manipulation, amygdala c-Fos protein expression was assessed and compared to the amounts of freezing observed.

2.1. Materials and Methods

2.1.1 Subjects

Male, Long-Evans rats (n = 42) from Charles River Canada (St. Constant, Québec) weighed 275 - 315 g at the beginning of the experiment. They were housed singly in hanging wire cages with water freely available in a temperature (23°C) and light (on at 700 h off at 1900 h) controlled room. All procedures were in accordance with guidelines of the Canadian Council on Animal Care and experimental protocols approved by the McGill University Animal Care Committee.

2.1.2 Apparatus

The main apparatus was a shuttle-box consisting of two adjacent stainless steel compartments with clear Plexiglas front walls and a connecting door in the center of the common wall. Each compartment measured 29 x 28 x 24 cm. The walls of the compartments were covered with self-adhesive plastic sheeting. One compartment was grey, the other was checkered black and white. The floors of both compartments consisted of stainless steel rods that could be connected to a shock generator. The rods were 0.5 cm in diameter and 1.5 cm apart. For each rat, one compartment served as the paired side (in which shock was given) and the other as the unpaired side (in which no shock was given). When a compartment was

unpaired, the rod floor was covered with 0.5 cm wire mesh. The shuttle-box rested on two stainless steel catch pans 6 cm below the rod floor.

A free-standing box (Box C) constructed with a wood frame (29 cm x 28 cm x 24 cm), 1.0 cm wire mesh walls and top, and a flat steel plate floor was also used. Box C was located inside a larger wooden box with an open front. Two 25 watt light bulbs hung inside the large box, one on each side of Box C.

2.1.3. Behavioral Procedure

The experiment began one week after the rats were delivered to the animal facility. During the experiment, all rats were fed 30 - 35 g of food each day at 1600 h, approximately 4 h after the end of the experimental procedures. All rats were handled for three consecutive days in the testing room. On each day, six to eight rats were placed for 2 h into a large (60 x 60 x 60 cm) open field wooden box with wood chips covering the floor. During this time each rat was picked up and held by the experimenter for 5 min.

On each of the two days following the handling period, each rat was exposed to Box C for 15 min per day in the testing room. On the following day, each rat was first placed into its randomly assigned paired compartment (rod floor exposed) for 6 min and then immediately placed into its unpaired compartment (wire mesh floor covering rods) for 6 min. No experimental events occurred during these pre-exposure sessions.

Shock training was given the following day. The rats were randomly assigned to a shock-stay group (n = 14), a shock-switch group (n = 14), a no shock-stay group (n = 10), and a no shock-switch group (n = 4). Rats in the shock-stay group were placed into their paired shuttle-box compartments. After 2 min, they received 2, 0.5 s, 1.0 mA foot shocks with a 1 min inter-shock interval. The rats stayed in the shock-paired compartment for an additional 6 min, during which an experimenter recorded the times at which the rats started and stopped freezing - defined as the absence of all body movement including the absence of whisker and nose movements [8,25]. These observations were used to calculate a percent freezing score (total s spent freezing x 100/ 360 s). Rats in the no shock-stay group were treated identically but did not receive shock.

Rats in the shock-switch group were placed into their paired compartment and

shocked in the same way as the rats in the shock-stay group. Immediately after the second shock the rats were moved from the shock-paired compartment to Box C by the experimenter. They remained there for 6 min while freezing was recorded. Rats in the no shock-switch group were treated identically but did not receive shock.

Approximately 90 min after shock training, the brains of a subset of the rats in each group (shock-switch = 8; shock-stay = 8; no shock-switch = 4; no shock-stay = 4) were prepared for c-Fos immunocytochemistry using methods described below.

Twenty-four hours later, the remaining rats in each group (shock-stay = 6; shock-switch = 6; no shock-stay = 6) were placed into their shock-paired compartments for 6 min. No shock was given and freezing was scored as described above.

2.1.4. c-Fos immunocytochemistry

Rats used for c-Fos immunocytochemistry received an i.p. injection of 65 mg/ml sodium pentobarbital and were perfused through the heart with 100 ml of a 0.9% saline/ heparin (1,000 units) solution over 5 min, followed by 400 ml of a 4% paraformaldehyde/ 0.1 M phosphate buffer (PB) solution (pH 7.4) infused at 20 ml/ min for 5 min, then at 15 ml/ min for 20 min. Brains were removed and post-fixed for 2 h in 4% paraformaldehyde and then stored in a 30% sucrose/ PB solution at 4° C for 48 h before being frozen in dry ice and sliced (25 μm) in a cryostat at -20° C through the rostral-caudal extent of the amygdala. Every third brain section was put into a sodium azide/ PB solution. These sections were washed in a 0.01 M phosphate buffered saline solution containing 0.002% Trition-X (PBS-TX). They were incubated at room temperature for 15 min in a 0.3% hydrogen peroxide/ PBS-TX solution. This was followed by 3 washes in PBS-TX and a 30 min incubation at room temperature in 3% normal goat serum (Vector)/ PBS-TX. The sections were washed once in PBS-TX then transferred to the primary antibody (polyclonal c-Fos Ab-5; lot # D09803; Calbiochem;1:50,000) and incubated for 48 h at 4° C.

Following primary antibody incubation, tissue sections were washed 3 times in PBS-TX and incubated for 2 h at room temperature in the secondary antibody (biotinylated anti-rabbit made in goat; Vector BA-1000). Sections were then washed 3 times in PBS-TX and incubated at room temperature in an avidin-biotin complex (ABC Elite Kit; Vector PK-6100)

for 1 h. After 3 more washes in PBS only, sections were developed with a nickel enhanced DAB solution and float-mounted on nickel chromium gel coated microscope slides. They were dehydrated in graded alcohols and cover slipped.

Brains from 6 additional male Long Evans rats used for an anatomical study were perfused and sliced through the rostral-caudal extent of the amygdala as described above. Sections were mounted on nickel chromium gel coated slides and stained for acetylcholinesterase (AchE) [73]. This stain produced good differentiation of the LA, BLA and CeA and less intense differentiation of the MeA (Figures 2A and 2A1). A section at 2.80 mm posterior to bregma [73] was taken from each of these brains and the MeA, BLA, CeA, and LA subregions were outlined using a microscope and imaging software (Scion Image).

c-Fos stained sections were examined through a microscope using 4x magnification. c-Fos positive cells were counted in each amygdala region by the experimenter, without knowledge of the experimental group to which each rat was assigned. The outlines from the AchE stained sections were used to restrict the cell counts to specific amygdala subregions.

Treating the data for each nucleus separately, the counts for each rat in the experiment were calculated as a percent of the mean counts of the rats in the no-shock switch and no-shock stay groups. This transformation gave means of 100% for the two no-shock groups while preserving an estimate of the variance of the individual scores around that mean. It also gave mean and variance estimates for the shock-stay and shock-switch groups that could be compared directly to the means of the no-shock control groups using standard ANOVA techniques. This procedure corrected for large differences in the baseline counts among the nuclei.

2.1.5. Results

Two rats (one each from the no shock-switch and no shock-stay groups) were removed from the statistical analyses as their brains were discovered to have atrophied hippocampuses. The statistical analyses were based on the remaining 22 rats.

2.1.6. Shock Training

Freezing during the immediate post-shock period is shown in Figure 1a. The rats in the shock-stay group froze more than the rats in the shock-switch and no-shock groups. A

one-way randomized ANOVA on the percent freezing scores revealed a main effect of group ($F(3,18) = 18.68$, $p < 0.001$). Fisher's LSD post-hoc analysis [60] showed that the mean freezing score for the shock-stay group was significantly higher than the mean scores for all other groups (t values > 4.80, $p < 0.01$). No other means were significantly different from each other.

Representative c-Fos stained sections are shown in Figure 2. Elevated c-Fos expression in the brains of the shock-switch and -stay rats is evident from a comparison with the brains of the no shock-switch and -stay rats. There was also a difference between the right and left hemispheres of the shocked rats.

The percent of control value scores for c-Fos positive cells are shown in Figure 3. A three-way repeated measures ANOVA with group as the between factor and amygdala region and hemisphere as repeated measures revealed significant main effects of group ($F(3,3) = 31.53$, $p < 0.0001$), region ($F(3,9) = 9.82$, $p < 0.0001$), and a significant interaction between these two factors ($F(9,72) = 3.58$, $p < 0.01$). There was also a significant main effect of hemisphere ($F(1,3) = 4.21$, $p < 0.05$).

Fisher's LSD post-hoc tests on the means for the MeA did not reveal any group differences in either hemisphere. In the left BLA there was significantly more c-Fos expression in the shock-switch than in the no-shock switch group ($t(9) = 3.11$, $p < 0.01$) but there was no significant difference between the shock-stay and no shock-stay groups ($t(9) < 1.0$). There were no differences among the means in the right BLA. In the CeA there was a significant elevation in c-Fos expression following shock in both the switch and stay conditions in the left and right hemispheres (t(9) values > 3.5, p values < 0.01). In the left LA c-Fos was significantly increased in both shock groups (t(9) values > 4.0, p values < 0.01) compared to their no-shock controls. In the right LA there was a significant increase in c-Fos expression for the shock-switch ($t(9) = 2.94$, $p < 0.01$), but not for the shock-stay ($t(9) = 1.71$) group.

2.1.7. Exposure to the aversive CS

Figure 1b shows the mean amounts of freezing observed during the 6 min following shock on the training day and during the test given 24 hr later for a subset of the rats. A two-

way repeated measures ANOVA (group by day) revealed significant main effects of group ($F(2,15) = 99.49$, $p < 0.001$) and day ($F(1,2) = 104.64$, $p < 0.001$), and a significant interaction ($F(2,15) = 46.41$, $p < 0.001$). Fisher's LSD tests showed that during the immediate post-shock period the rats in the shock-stay group froze significantly more than the rats in the both the shock-switch ($t(10) = 8.66$, $p < 0.01$) and no shock-stay ($t(10) = 9.37$, $p < 0.01$) groups. During the test 24 hours later, the rats in both the shock-stay ($t(10) = 13.44$, $p < 0.01$) and shock switch ($t(10) = 14.01$, $p < 0.01$) groups (which did not differ) froze significantly more than the rats in the no shock-stay group.

2.1.8. Experiment 1 Discussion

Rats that were switched from the shock compartment to a neutral compartment immediately after shock froze much less than rats that remained in the shock compartment. This finding replicates previous reports [7,8,25] and indicates that post-shock freezing may primarily be elicited by the shock context. Exposure to the shock compartment 24 hours later resulted in equivalent, elevated freezing in both the shock-switch and -stay groups compared to a no shock control group. Thus freezing during a test 24 hours later is not affected by whether or not the rats froze during or immediately after training [see also 64].

c-Fos expression was elevated by similar amounts in the left and right CeA when the rats froze following shock (shock-stay condition) and when they did not freeze (shock-switch condition). This finding is consistent with previous reports [66,77]; however the present finding suggests that CeA c-Fos protein expression is unrelated to freezing. Rather, elevated c-Fos expression in the CeA appears to have been produced by the shock, an unconditioned stimulus.

c-Fos expression in both the left and right LA was also elevated following shock, regardless of whether or not the rats froze during the post-shock period. Previous studies have not found elevated protein or gene expression in the LA following shock [78,81]. Because the absolute number of c-Fos activated cells in LA was much lower than in other parts of the amygdala, an overall statistical analysis comparing raw counts from all parts of the amygdala failed to detect the increase in activation in that region. However, as shown by the percent change from baseline score used in the present study, there was a highly

significant change in c-Fos expression following shock in the LA. This could account for discrepancies reported in the literature.

The dissociation between freezing and c-Fos expression suggests that there is a population of neurons in the CeA and LA that is activated by exposure to an aversive US, but that this activation is not always related to the occurrence of freezing. The fact that the activation occurred in both the shock-stay and shock-switch conditions suggests that it may be a result of exposure to the US.

In this study, as in some previous reports [66,77] c-Fos expression in MeA was not elevated following exposure to shock. In other reports [74,77,78,81] increased c-Fos in the MeA was observed following shock. A possible explanation for this discrepancy is suggested by evidence that exposure to novel environments elevates c-Fos expression in the MeA [43,81], which disappears with repeated exposures in some [14,43,77] but not all [13] cases. Procedures that do not include pre-exposure to the context may fail to detect an effect of shock because expression is elevated in the control group. Thus, Pezzone, et al. [74], Radulovic, et al. [77; condition B], and Ressler, et al. [78] gave extended pre-exposure to the contextual cues and reported elevated c-Fos expression in MeA following shock, while Milanovic, et al. [66] and Radulovic, et al. [77; condition A] gave less preexposure to the conditioning chamber and did not find elevated MeA c-Fos expression following shock.

The present experiments may have failed to detect an effect of shock on MeA c-Fos expression because the procedure included only a single 6 min session of exposure to the shock context, significantly less that used in experiments that found such increases [74,77,78].

In the BLA, c-Fos labeling was not consistently elevated following shock, as has been previously reported [66,74,77,81].

3. Experiment 2

This experiment examined the pattern of amygdala c-Fos expression following exposure to a contextual CS. Rats received shock in one (paired) compartment of a shuttle-box on one day and no shocks in the other (unpaired) compartment on the next day. Twenty-four hours later half of these rats were exposed to the paired and the other half to the unpaired compartment. Freezing and c-Fos expression were assessed and compared.

3.1. Materials and Methods

The Subjects were 24 rats similar to those used in Experiment 1. The shuttle-box and Box C were the same as in Experiment 1.

The adaptation and handling procedures were the same as described in Experiment 1. For shock training, 16 rats were placed into their paired compartments. After 2 min they were given 4, 0.5 sec, 1.0 mA foot shocks with a 1 min inter-shock interval, for a total of 6 min. The remaining 8 rats were placed into their paired compartments for 6 min but were not given any shocks. On the following day, all rats were placed into their unpaired compartments for 6 min with no shocks. Freezing was measured during both of these sessions.

All rats were tested 24 hours later. The 16 shocked rats were randomly assigned to either the shock-paired condition (Sh-P; n = 8) and were placed into their paired compartments, or to the shock-unpaired condition (Sh-U; n = 8) and were placed into their unpaired compartments. Rats that were not shocked on the shock-training day (NSh-P; n = 8) were placed into their paired compartments. Freezing was measured for 6 min in all groups.

Approximately 90 min after the end of this test, brain tissue from all rats in the experiment was processed for c- Fos protein expression and analyzed as described in Experiment 1.

3.1.1. Results Experiment 2

The freezing data are shown in Figure 4. The rats in group Sh-P froze more than the rats in group Sh-U, and the rats in both of these groups froze more than the rats in group NSh-P during the test. A two-way repeated measures ANOVA (group by day) revealed a significant main effect of group ($F(2,21) = 81.61$, $p < 0.001$) and a significant interaction ($F(2,21) = 7.89$, $p < 0.01$) between group and day. Fisher's LSD tests showed that on the shock training day, freezing was significantly higher in both groups Sh-P and Sh-U than in group NSh-P ($t(14)$ values > 5.0, $p < 0.01$). On the test day, group Sh-P froze significantly more than groups Sh-U ($t(14) = 4.07$, $p < 0.01$) and NSh-P ($t(14) = 10.07$, $p < 0.01$). The rats in group Sh-U also froze significantly more than the rats in group NSh-P ($t(14) = 6.0$, p

< 0.01).

The difference in freezing between groups Sh-P and Sh-U shows that the contextual cues in the rats' paired compartments functioned as a CS. The difference in freezing between groups Sh-U and NSh-P shows that other cues in the experimental environment also elicited freezing, although somewhat less effectively than the explicitly paired cues.

Figure 5 shows brain sections stained for c-Fos following testing. Amygdala subregions in both hemispheres of rats in group Sh-P (Figs 5c and 5c1) had higher numbers of c-Fos positive cells than rats in groups Sh-U (Figs 5b and 5b1) and NSh-P (Figs 5a and 5a1).

The c-Fos data are shown in Figure 6. One brain from each group was excluded from the c-Fos analysis due to improper staining, leaving 7 rats in each group. The three way, repeated measures ANOVA using group as the between factor and amygdala region and hemisphere as repeated measures revealed significant main effects of group ($F(2,3) = 32.41$, $p < 0.0001$) and region ($F(3,6) = 6.72$, $p < 0.001$) and a significant interaction between these two factors ($F(6,72) = 5.71$, $p < 0.0001$). There was also a main effect of hemisphere ($F(1,2) = 10.23$, $p < 0.01$).

Post-hoc analysis of the data for MeA did not reveal any significant differences among the groups in either hemisphere. In both the left and right hemispheres of the BLA there were significantly more c-Fos positive cells in group Sh-P than in groups Sh-U ($t(12)$ values > 3.0, $p < 0.01$) and NSh-P ($t(12)$ values >3.0, $p < 0.01$). The mean number of positive cells in group Sh-U was significantly higher than in group NSh-P in the left ($t(12) = 2.85$, $p < 0.01$) but not the right ($t(12) < 1.0$) BLA.

In both the left and right CeA there were significantly more c-Fos positive cells in group Sh-P than in groups Sh-U ($t(12)$ values > 6.0, $p < .01$) and NSh-P ($t(12)$ values > 6.0, $p < .01$), but there were no significant differences between the means for the two latter groups ($t(12)$ values < 1.0). In both the left and right LA, there were significantly more positive cells in group Sh-P than in groups Sh-U ($t(12)$ values > 3.5, $p < 0.01$) and NSh-P ($t(12)$ values >3.5, $p < 0.01$), but there were no significant differences between the means for the two latter groups ($t(12)$ values < 2.0).

3.1.2. Experiment 2 Discussion

Higher levels of freezing and c-Fos expression in the CeA, BLA, and LA were observed when rats were exposed to a compartment in which they had been shocked compared to exposure to a distinct compartment in which they had not been shocked. The rats in the latter group froze more than rats that had never been shocked, but these two groups showed similar levels of c-Fos expression. These findings suggest that amygdala c-Fos expression and freezing may be related in the presence of a highly effective (explicitly paired) CS, but that freezing in the presence of less effective contextual cues is not related to similar amygdala c-Fos expression. This indicates that freezing in the presence of "background" contextual cues [see 76] does not depend on a form of neural activation detected by c-Fos expression in CeA, BLA, and LA.

Exposure to the paired but not to the unpaired context elevated c-Fos expression in the CeA in both the left and right hemispheres. These findings are consistent with those of several other studies showing that neurons in the CeA are activated by CSs explicitly paired with shock [40,66,88].

The right and left BLA also showed elevated c-Fos expression following exposure to the contextual CS. In other studies, BLA activation was increased following exposure to a paired [88] but not to an unpaired [40] auditory CS even though the unpaired CS elicited freezing for 20% of the observation period [40]. Milanovic, et al. [66] did not report elevated BLA c-Fos expression during exposure to a contextual CS. This could have been due to the use of lower shock levels (1, 0.7 mA shock) on the conditioning trials than were used in the studies that found elevated expression; [88] used 2, 1.0 mA shocks; [40] used 5, 0.45 mA shocks; the present study used 4, 1.0 mA shocks. It appears that a minimum shock level during contextual conditioning or the use of an explicit cue may be required to observe elevations in BLA c-Fos expression during testing. This may suggest that activity levels in weakly or inexplicitly conditioned rats do not reach a threshold required for detection by c-Fos expression, or that strong conditioning is mediated by a population of BLA neurons and weak conditioning is mediated by a different population of neurons, either in BLA or elsewhere.

The left BLA showed elevated c-Fos expression following exposure to the unpaired context. This suggests that a population of left BLA neurons may mediate weak conditioning. Alternatively, since the activation observed in the left BLA in the paired and unpaired conditions coincided with the amount of freezing observed, it suggests that the function of the activated neurons in this region may be directly related to the production of freezing.

The elevated c-Fos labeling in the LA following exposure to conditioned cues has not been a consistent finding [40,81]. As discussed in Experiment 1 this could be due to the use of a percent change score in the present study. This analysis made it possible to observe changes in this region even though its baseline level of c-Fos expression was much lower than that of the other amygdala regions.

Exposure to the contextual CS did not elevate c-Fos expression in the MeA. This is consistent with previous reports [66,81] and suggests that the MeA does not contain c-Fos expressing neurons that are activated by exposure to shock-paired contextual CSs or by freezing.

In Experiment 2 freezing and c-Fos expression in three amygdala regions were dissociated to some extent. A population of amygdala neurons was activated by exposure to a contextual CS, but freezing occurred when these neurons were not activated, or not activated to the same degree or in the same way as when the rat was exposed to the CS. This finding leads to the conclusion that the activity of these neurons may be controlled by the CS and is, at best, indirectly related to freezing [see also 9,66].

4. General Discussion

In Experiment 1 c-Fos expression was elevated in the CeA and LA in both the shock-switch and -stay groups but only the shock-stay group showed high levels of freezing immediately after shock. This suggests either that c-Fos expression following shock overshadowed any activation related to the occurrence of freezing or that the activation occurred independently of freezing. If the activation produced by exposure to the US was independent of freezing there are two possibilities. The activation could have been produced by the aversive (or some other) property of the shock [see 4,66,66] or, since both groups displayed freezing when re-exposed to the contextual CS 24 hours later, the post-shock

activation could have resulted from the acquisition of a representation of the US. During the test, this US representation would have been activated by the contextual CS.

In Experiment 2, using a differential conditioning paradigm, high levels of freezing were observed during exposure to the explicitly paired contextual CS and moderate levels of freezing occurred during exposure to the unpaired context. The rats in both groups underwent the same conditioning procedure but were exposed to different sets of cues during the test. The BLA, CeA, and LA showed elevated c-Fos expression following exposure to the paired but not the unpaired context [see also 40,66,88]. C-Fos expression during exposure to the paired contextual CS may indicate activation of a US representation by the CS.

Previous reports have found that amygdala activity is sensitive to presentation of unconditioned aversive events. Handling rats for the first time elevated amygdala *c-fos* mRNA [14,77]. Exposure to an unconditioned 20 kHz tone elevated c-Fos expression in both the BLA and CeA [4] in the absence of any learning. In a delayed shock procedure [66,77] CeA c-Fos labeling was elevated in both the immediate and delayed shock conditions, but only the mice in the delayed condition froze 24 hours later, indicating that learning had occurred in this group. These findings suggest that c-Fos expressing neurons in the amygdala are sensitive to unconditioned aversive events [91] in the absence of learning that is reflected in behavioral changes. It may be the case that c-Fos expressing neurons mediate a representation of the US rather than an association between the CS and US.

Induction of the c-Fos protein represents a small fraction of the total number of proteins expressed in neurons [18]. Expression of *zif268* [39,78,81], phosphorylated CREB [40], or many other immediate early genes [78,90,91] occur in the amygdala following aversive conditioning procedures. These and other neural events (e.g., LTP) in the amygdala appear to be sensitive to the formation of associations between the CS and US [78] while c-Fos expression does not [13]. In the case of c-Fos, the association between the CS and US may occur between brain regions with the amygdala supporting the US representation and, in the case of contextual conditioning, a neural system that includes the hippocampus supporting the CS representation [see 66].

Neurons in the left BLA expressed c-Fos following the shock-switch manipulation (Experiment 1) and following exposure to the unpaired cues (Experiment 2). Freezing was lower in these groups than in the shock-stay and shock-paired groups, respectively. This suggests that activity in neurons expressing c-Fos in the left BLA may be associated with reductions in freezing [see also 44]. Coleman-Mesches and McGaugh [19,20] found that muscimol injections into the right but not left BLA blocked retention of inhibitory avoidance of which freezing is a major component. This treatment would leave the left BLA active, contributing to a reduction in freezing and, therefore, to a reduction in inhibitory avoidance latencies. Activation of left BLA neurons might be associated with behavioral activation leading to reduced freezing and impaired passive avoidance. This conclusion is consistent with that suggested by Holahan & White [44] and others [12,11] indicating an inverse relationship between BLA activity and freezing.

There did not appear to be any consistent relationships between c-Fos induction in the CeA or LA and freezing. If activation of these neurons does not produce freezing, what does it do? One possibility is that the c-Fos expressing neurons in the CeA and LA are part of a neural circuit that produces an aversive affective state. Aversive states are thought to arise during exposure to unconditioned and conditioned aversive stimuli and to promote the expression of behaviors such as freezing and avoidance [10,21,52,67,69,69,70].

The present findings suggest the hypothesis that a population of c-Fos expressing neurons in the CeA and LA mediate a representation of the US during aversive conditioning. Activation of this representation by the US or CS results in the production of a set of physiological responses contributing to an aversive affective state ["fear"; 22,27,52,58]. Information concerning the contextual CS may be processed by the hippocampus and contribute to the representation of the CS available to the amygdala or directly to the observed freezing [2,65]. Projections from the amygdala to the central gray are involved in the production of freezing [16,17,54].

It is also possible that representations of the CS processed by the hippocampus and the amygdala converge on neurons in the dorsal striatum [37,48,49,63] and activation of this structure contributes to freezing [3,84,96]. In this case, the dorsal striatum may mediate an

association between the CS representation and the freezing behavior resulting in elevated freezing. This possibility requires further examination.

References

[1] Angle,P. and Karin,M., The role of Jun, Fos and the AP-1 complex in cell proliferation and transformation. Biochimica et Biophysica Acta 1991; 1072: 129-157.

[2] Bach,M.E., Hawkins,R.D., Osman,M., Kandel,E.R., and Mayford,M., Impairment of spatial but not contextual memory in CAMKII mutant mice with selective loss of hippocampal LTP in the range of the theta frequency. Cell 1995; 81: 905-915.

[3] Beck,C.H.M. and Fibiger,H.C., Conditioned fear-induced changes in behavior and in the expression of the immediate early gene *c-fos*: with and without diazepam pretreatment. J. Neurosci. 1995; 15: 709-720.

[4] Beckett,S.R.G., Duxon,M.S., Aspley,S., and Marsden,C.A., Central c-Fos expression following 20kHz/ultrasound induced defence behaviour in the rat. Brain Res. Bull. 1997; 42: 421-426.

[5] Bindra,D. and Anchel,H., Immobility as an avoidance response, and its disruption by drugs. Journal of the Experimental Analysis of Behavior 1963; 6: 213-218.

[6] Blanchard,D.C. and Blanchard,R.J., Innate and conditioned reactions to threat in rats with amygdaloid lesions. J. Comp. Physiol. Psychol. 1972; 81: 281-290.

[7] Blanchard,R.J. and Blanchard,C., Crouching as an index of fear. J. Comp. Physiol. Psychol. 1969; 67: 370-375.

[8] Bolles,R.C. and Collier,A.C., The effect of predictive cues on freezing in rats. Anim. Learn. Behav. 1976; 4: 6-8.

[9] Boujabit,M., Bontempi,B., Destrade,C., and Gisquet-Verrier,P., Exposure to a retrieval cue in rats induces changes in regional brain glucose metabolism in the amygdala and other related brain structures. Neurobiol. Learn. Mem. 2003; 79: 57-71.

[10] Brown,J.S. and Jacobs,A., The role of fear in the motivation and acquisition of responses. J. Exp. Psychol. 1949; 39: 747-759.

[11] Cahill,L., Vazdarjanova,A., and Setlow,B., The basolateral complex is involved with, but is not necessary for, rapid acquisition of Pavlovian 'fear conditioning'. Eur. J. Neurosci. 2000; 12: 3044-3050.

[12] Cahill,L., Weinberger,N.M., Roozendaal,B., and McGaugh,J.L., Is the amygdala a

locus of "conditioned fear"? Some questions and caveats. Neuron 1999; 23: 327-328.

[13] Campeau,S., Falls,W.A., Cullinan,W.E., Helmreich,D.L., Davis,M., and Watson,S.J., Elicitation and reduction of fear: behavioural and neuroendocrine indices and brain induction of the immediate-early gene *c-fos*. Neuroscience 1997; 78: 1087-1104.

[14] Campeau,S., Hayward,M.D., Hope,B.T., Rosen,J.B., Nestler,E.J., and Davis,M., Induction of the *c-fos* proto-oncogene in rat amygdala during unconditioned and conditioned fear. Brain Res. 1991; 565: 349-352.

[15] Carew,T.J., Molecular memory enhancement of memory formation. Neuron 1996; 16: 5-8.

[16] Carrive,P., Leung,P., Harris,J., and Paxinos,G., Conditioned fear to context is associated with increased Fos expression in the caudal ventrolateral region of the midbrain periacqueductal gray. Neuroscience 1997; 78: 165-177.

[17] Carrive,P., The periacqueductal gray and defensive behavior: functional representation and neuronal organization. Behav. Brain Res. 1993; 58: 27-47.

[18] Clayton,D.F., The genomic action potential. Neurobiol. Learn. Mem. 2000; 74: 185-216.

[19] Coleman-Mesches,K. and McGaugh,J.L., Differential effects of pretraining inactivation of the right or left amygdala on retention of inhibitory avoidance training. Behav. Neurosci. 1995; 109: 642-647.

[20] Coleman-Mesches,K. and McGaugh,J.L., Muscimol injected into the right or left amygdaloid complex differentially affects retention performance following aversively motivated training. Brain Res. 1995; 676: 183-188.

[21] Davis,M., Neurobiology of fear responses: the role of the amygdala. J. Neuropsychiatry Clin. Neurosci. 1997; 9: 382-402.

[22] Davis,M., The role of the amygdala in conditioned and unconditioned fear and anxiety. In: Aggleton,J.P. (Ed.), The Amygdala: Second Edition: A Functional Analysis, Oxford: Oxford University Press, 2000, pp. 213-287.

[23] Dielenberg,R.A., Hunt,G.E., and McGregor,I.A., 'When a rat smells a cat': the distribution of Fos immunoreactivity in rat brain following exposure to a predatory

odor. Neuroscience 2001; 104: 1085-1097.

[24] Everitt,B.J., Cardinal,R.N., Hall,J., Parkinson,J.A., and Robbins,T.W., Differential involvement of amygdala subsystems in appetitive conditioning and drug addiction. In: Aggleton,J.P. (Ed.), The Amygdala: Second Edition: A Functional Analysis, Oxford: Oxford University Press, 2000, pp. 353-390.

[25] Fanselow,M.S., Conditional and unconditional components of postshock freezing. Pav. J. Biol. Sci. 1980; 15: 177-182.

[26] Fanselow,M.S., The postshock activity burst. Anim. Learn. Behav. 1982; 10: 448-454.

[27] Fanselow,M.S., What is conditioned fear? Trends. Neurosci. 1984; 7: 460-462.

[28] Fanselow,M.S. and Kim,J.J., Acquisition of contextual Pavlovian fear conditioning is blocked by application of an NMDA receptor antagonist D,L-2-Amino-5-Phosphonovaleric Acid to the basolateral amygdala. Behav. Neurosci. 1994; 108: 210-212.

[29] Fanselow,M.S., Landeira-Fernandez,J., DeCola,J.P., and Kim,J.J., The immediate-shock deficit and postshock analgesia: implications for the relationship between the analgesic CR and UR. Anim. Learn. Behav. 1994; 22: 72-76.

[30] Fanselow,M.S. and LeDoux,J.E., Why we think plasticity underlying Pavlovian fear conditioning occurs in the basolateral amygdala. Neuron 1999; 23: 229-232.

[31] Fendt,M., Injections of the NMDA receptor antagonist aminophosphonopentanoic acid into the lateral nucleus of the amygdala block the expression of fear-potentiated startle and freezing. J. Neurosci. 2001; 21: 4111-4115.

[32] Fendt,M. and Fanselow,M.S., The neuroanatomical and neurochemical basis of conditioned fear. Neurosci. Biobehav. Rev. 1999; 23: 743-760.

[33] Freeman,F.M. and Rose,S.P.R., Expression of Fos and Jun proteins following passive avoidance training in the day-old chicks. Learn. Mem. 2000; 6: 389-397.

[34] Gallagher,M., The amygdala and associative learning. In: Aggleton,J.P. (Ed.), The Amygdala: Second Edition: A Functional Analysis, Oxford: Oxford University Press, 2000, pp. 311-329.

[35] Goelet,P., Castellucci,V.F., Schacher,S., and Kandel,E.R., The long and the short of long-term memory - a molecular framework. Nature 1986; 322: 419-422.

[36] Goosens,K.A. and Maren,S., Contextual and auditory fear conditioning are mediated by the lateral, basal, and central amygdaloid nuclei in rats. Learn. Mem. 2001; 8: 148-155.

[37] Groenewegen,H.J., Vermeulen-Van der Zee,E., te Kortschot,A., and Witter,M.P., Organization of the projections from the subiculum to the ventral striatum in the rat. A study using anterograde transport of *Phaseolus vulgaris* leucoagglutinin. Neuroscience 1987; 23: 103-120.

[38] Guzowski,J.F., Insights into immediate-early gene function in hippocampal memory consolidation using antisense oligonucleotide and fluorescent imaging approaches. Hippocampus 2002; 12: 86-104.

[39] Hall,J., Thomas,K.L., and Everitt,B.J., Cellular imaging of *zif268* expression in the hippocampus and amygdala during contextual and cued fear memory retrieval: selective activation of hippocampal CA1 neurons during the recall of contextual memories. J. Neurosci. 2001; 21: 2186-2193.

[40] Hall,J., Thomas,K.L., and Everitt,B.J., Fear memory retrieval induces CREB phosphorylation and Fos expression within the amygdala. Eur. J. Neurosci. 2001; 13: 1453-1458.

[41] Helmstetter,F.J. and Bellgowan,P.S., Effects of muscimol applied to the basolateral amygdala on acquisition and expression of contextual fear conditioning in rats. Behav. Neurosci. 1994; 108: 1005-1009.

[42] Herrera,D.G. and Robertson,H.A., Activation of *c-fos* in the brain. Prog. Neurobiol. 1996; 50: 83-107.

[43] Hess,U.S., Gall,C.M., Granger,R., and Lynch,G., Differential patterns of *c-fos* mRNA expression in amygdala during successive stages of odor discrimination learning. Learn. Mem. 1997; 4: 262-283.

[44] Holahan,M.R. and White,N.M., Conditioned memory modulation, freezing, and avoidance as measures of amygdala-mediated conditioned fear. Neurobiol. Learn.

Mem. 2002; 77: 250-275.

[45] Kaczmarek,L., Molecular biology of vertebrate learning: is *c-fos* a new beginning? J. Neurosci. Res. 1993; 34: 377-381.

[46] Kaczmarek,L. and Chaudhuri,A., Sensory regulation of immediate-early gene expression in mammalian visual cortex: implications for functional mapping and neural plasticity. Brain Res. Rev. 1997; 23: 237-256.

[47] Kaczmarek,L. and Nikolaev,E., C-Fos protooncogene expression and neuronal plasticity. Acta Neurobiol. Exp. 1990; 50: 173-179.

[48] Kelley,A.E. and Domesick,V.B., The distribution of the projection from the hippocampal formation to the nucleus accumbens in the rat: an anterograde- and retrograde-horseradish peroxidase study. Neuroscience 1982; 7: 2321-2335.

[49] Kelley,A.E., Domesick,V.B., and Nauta,W.J.H., The amygdalostriatal projection in the rat - an anatomical study by anterograde and retrograde tracing methods. Neuroscience 1982; 7: 615-630.

[50] Kiernan,M.J., Westbrook,R.F., and Cranney,J., Immediate shock, passive avoidance, and potentiated startle: implications for the unconditioned response to shock. Anim. Learn. Behav. 1995; 23: 22-30.

[51] Kim,J.J., Rison,R.A., and Fanselow,M.S., Effects of amygdala, hippocampus, and periacqueductal gray lesions on short- and long-term contextual fear. Behav. Neurosci. 1993; 107: 1093-1098.

[52] LeDoux,J., The amygdala and emotion: a view through fear. In: Aggleton,J.P. (Ed.), The Amygdala: Second Edition: A Functional Analysis, Oxford: Oxford University Press, 2000, pp. 289-310.

[53] LeDoux,J.E., Emotional networks and motor control: a fearful view. In: Holstege,G., Bandler,R., Saper,C.B. (Eds.), Prog. Brain Res., Elsevier Science, 1996, pp. 437-446.

[54] LeDoux,J.E., Iwata,J., Cicchetti,P., and Reiss,D.J., Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlate of conditioned fear. J. Neurosci. 1988; 8: 2517-2529.

[55] Lee,H.J., Choi,J.-S., Brown,T.H., and Kim,J.J., Amygdalar NMDA receptors are

critical for the expression of multiple conditioned fear responses. J. Neurosci. 2001; 21: 4116-4124.

[56] Lee,Y., Walker,D.L., and Davis,M., Lack of a temporal gradient of retrograde amnesia following NMDA-induced lesions of the basolateral amygdala assessed with the fear-potentiated startle paradigm. Behav. Neurosci. 1996; 110: 836-839.

[57] Maren,S., Neurotoxic basolateral amygdala lesions impair learning and memory but not the performance of conditional fear in rats. J. Neurosci. 1999; 19: 8696-8703.

[58] Maren,S., Neurobiology of Pavlovian fear conditioning. Annu. Rev. Neurosci. 2001; 24: 897-931.

[59] Maren,S., Aharonov,G., Stote,D.L., and Fanselow,M.S., *N*-Methyl-D-Aspartate receptors in the basolateral amygdala are required for both acquisition and expression of conditional fear in rats. Behav. Neurosci. 1996; 110: 1365-1374.

[60] Maxwell,S.E. and Delaney,H.D., Designing experiments and analyzing data. Pacific Grove, CA: Brooks/Cole Publishing Co., 1990, 206 pp.

[61] McAllister,W.R. and McAllister,D.E., Behavioral measurement of conditioned fear. In: Brush,F.R. (Ed.), Aversive Conditioning and Learning. New York: Academic Press, 1971, pp. 105-179.

[62] McGaugh,J.L., Ferry,B., Vazdarjanova,A., and Roozendaal,B., Amygdala: role in modulation of memory storage. In: Aggleton,J.P. (Ed.), The Amygdala: Second Edition: A Functional Analysis, Oxford: Oxford University Press, 2000, pp. 391-423.

[63] McGeorge,A.J. and Faull,R.L.M., The organization of the projection from the cerebral cortex to the striatum in the rat. Neuroscience 1989; 29: 503-537.

[64] McNally,G.P., Gorrisen,M.C., Low,L.F., and Westbrook,R.F., Effects of contextual cues previously paired with footshock or illness on behavior and pain sensitivity in the rat. Anim. Learn. Behav. 1999; 27: 416-425.

[65] McNish,K.A., Gewirtz,J.C., and Davis,M., Evidence of contextual fear after lesions of the hippocampus: a disruption of freezing but not fear-potentiated startle. J. Neurosci. 1997; 17: 9353-9360.

[66] Milanovic,S., Radulovic,J., Laban,O., Stiedl,O., Henn,F., and Spiess,J., Production

of the Fos protein after contextual fear conditioning of C57BL/6N mice. Brain Res. 1998; 784: 37-47.

[67] Miller,N.E., Studies of fear as an acquirable drive: I. Fear as motivation and fear-reduction as reinforcement in the learning of new responses. J. Exp. Psychol. 1948; 38: 89-101.

[68] Morgan,J.I. and Curran,T., Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes *fos* and *jun*. Annu. Rev. Neurosci. 1991; 14: 421-451.

[69] Mowrer,O.H., On the dual nature of learning -- A re-interpretation of "conditioning" and "problem-solving". Harv. Edu. Rev. 1947; 17: 102-148.

[70] Mowrer,O.H. and Lamoreaux,R.R., Fear as an intervening variable in avoidance conditioning. J. Comp. Psychol. 1946; 39: 29-50.

[71] Muller,J., Corodimas,K.P., Fridel,Z., and LeDoux,J.E., Functional inactivation of the lateral and basal nuclei of the amygdala by muscimol infusion prevents fear conditioning to an explicit conditioned stimulus and to contextual stimuli. Behav. Neurosci. 1997; 111: 683-691.

[72] Nikolaev,E., Kaminska,B., Tischmeyer,W., Matthies,H., and Kaczmarek,L., Induction of expression of genes encoding transcription factors in the rat brain elicited by behavioral training. Brain Res. Bull. 1992; 28: 479-484.

[73] Paxinos,G. and Watson,C., The rat brain atlas in stereotaxic coordinates. New York: Academic Press, 1998.

[74] Pezzone,M.A., Lee,W.-S., Hoffman,G.E., and Rabin,B.S., Induction of c--Fos immunoreactivity in the rat forebrain by conditioned and unconditioned aversive stimuli. Brain Res. 1992; 597: 41-50.

[75] Phillips,R.G. and LeDoux,J.E., Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behav. Neurosci. 1992; 106: 274-285.

[76] Phillips,R.G. and LeDoux,J.E., Lesions of the dorsal hippocampal formation interfere with background but not foreground contextual fear conditioning. Learn. Mem. 1994;

1: 34-44.

[77] Radulovic,J., Kammermeier,J., and Spiess,J., Relationship between Fos production and classical fear conditioning: effects of novelty, latent inhibition, and unconditioned stimulus preexposure. J. Neurosci. 1998; 18: 7452-7461.

[78] Ressler,K.J., Paschall,G., Zhou,X., and Davis,M., Regulation of synaptic plasticity genes during consolidation of fear conditioning. J. Neurosci. 2002; 22: 7892-7902.

[79] Rose,S.P.R., How chicks make memories: the cellular cascade from *c-fos* to dendritic remodelling. Trends. Neurosci. 1991; 14: 390-397.

[80] Rose,S.P.R., Cell adhesion molecules and the transition from short- to long-term memory. J. Physiol. (Paris) 1996; 90: 387-391.

[81] Rosen,J.B., Fanselow,M.S., Young,S.L., Sitcoske,M., and Maren,S., Immediate-early gene expression in the amygdala following footshock stress and contextual fear conditioning. Brain Res. 1998; 796: 132-142.

[82] Sacchetti,B., Lorenzini,C.A., Baldi,E., Tassoni,G., and Bucherelli,C., Memorization of contextual and CS conditioned fear response (freezing) in a one-trial acquisition paradigm. Arch. Ital. Biol. 1999; 137: 235-248.

[83] Sagar,S.M., Sharp,F.R., and Curran,T., Expression of *c-fos* protein in brain: metabolic mapping at the cellular level. Science 1988; 240: 1328-1331.

[84] Salinas,J.A. and White,N.M. Differential contributions of the hippocampus, amygdala, and dorsal striatum to contextual and cued Pavlovian fear conditioning. *In press* Behav. Brain Res, 2003.

[85] Sander,G., Oberling,P., Silveira,M.C., Di Scala,G., Rocha,B., Bagri,A., and Depoortere,R., What brain structures are active during emotions? Effects of brain stimulation elicited aversion on *c-fos* immunoreactivity and behavior. Behav. Brain Res. 1993; 58: 9-18.

[86] Sheng,M. and Greenberg,M.E., The regulation and function of *c-fos* and other immediate early genes in the nervous system. Neuron 1990; 4: 477-485.

[87] Smith,M.A., Banerjee,S., Gold,P.W., and Glowa,J., Induction of *c-fos* mRNA in rat brain by conditioned and unconditioned stressors. Brain Res. 1992; 578: 135-141.

[88] Sotty,F., Sander,G., and Gosselin,O., Latent inhibition in conditioned emotional response: *c-fos* immunolabelling evidence for brain areas involved in the rat. Brain Res. 1996; 737: 243-254.

[89] Stanciu,M., Radulovic,J., and Spiess,J., Phosphorylated cAMP response element binding protein in the mouse brain after fear conditioning: relationship to Fos production. Mol. Brain Res. 2001; 94: 15-24.

[90] Stork,O. and Pape,H.-C., Fear memory and the amygdala: insights from a molecular perspective. Cell. Tiss. Res. 2002; 310: 271-277.

[91] Stork,O., Stork,S., Pape,H.-C., and Obata,K., Identification of genes expressed in the amygdala during the formation of fear memory. Learn. Mem. 2001; 8: 209-219.

[92] Tischmeyer,W. and Grimm,R., Activation of immediate early genes and memory formation. Cell Mol. Life Sci. 1999; 55: 564-574.

[93] Vazdarjanova,A., Cahill,L., and McGaugh,J.L., Disrupting basolateral amygdala function impairs unconditioned freezing and avoidance in rats. Eur. J. Neurosci. 2001; 14: 709-718.

[94] Vazdarjanova,A. and McGaugh,J.L., Basolateral amygdala is not critical for cognitive memory of contextual fear conditioning. Proc. Natl. Acad. Sci. U. S. A. 1998; 95: 15003-15007.

[95] Vazdarjanova,A. and McGaugh,J.L., Basolateral amygdala is involved in modulating consolidation of memory for classical fear conditioning. J. Neurosci. 1999; 19: 6615-6622.

[96] Viaud,M.D. and White,N.M., Dissociation of visual and olfactory conditioning in the neostriatum of rats. Behav. Brain Res. 1989; 32: 31-42.

[97] Wilensky,A.E., Schafe,G.E., and LeDoux,J.E., Functional inactivation of the amygdala before but not after auditory fear conditioning prevents memory formation. J. Neurosci. 1999; 19: 1-5.

[98] Wilensky,A.E., Schafe,G.E., and LeDoux,J.E., The amygdala modulates memory consolidation of fear-motivated inhibitory avoidance learning but not classical fear conditioning. J. Neurosci. 2000; 20: 7059-7066.

Figure legends

Figure 1. A) Freezing following shock. Mean observed freezing (± SEM) following shock and switch to a neutral compartment (Sh-Sw), shock and stay in the shock compartment (Sh-St), no shock and switch (NoSh-Sw) and no shock and stay (NoSh-St). ** $p < 0.01$ vs all other groups. B) Freezing during 24 hour re-test. Mean observed freezing (± SEM) on the shock-training day (abbreviations as used in A) and during the 6 min test in the shock-paired compartment. ** $p < 0.01$ Sh-St vs Sh-Sw and NoSh-St on training day; ++ $p < 0.01$ Sh-St and Sh-Sw vs NoSh-St on testing day.

Figure 2. A and A1) Acetylcholinesterase-stained section used for outline of amygdala regions (MeA - medial amygdala, BLA - basolateral amygdala, CeA - central amygdala, LA - lateral amygdala). A) Right hemisphere A1) Left hemisphere Figures B, B1, C, and C1 are digitized c-Fos-stained sections with the amygdala regions outlined. B) Right hemisphere of a no shock-stay rat. B1) Left hemisphere of a no shock-switch rat. B) Right hemisphere of a shock-stay rat. B1) Left hemisphere of a shock-switch rat.

Figure 3. Mean percentage of control of c-Fos positive cells (± SEM) in each amygdala region (abbreviations as in Fig 2) for the experimental groups (abbreviations as in Fig 1). The mean raw cell counts for the NSh-Sw group were: left MeA 81.3, BLA 23, CeA 22.3, LA 6 and right MeA 44.3, BLA 27.3, CeA 14.3, LA 5; the raw cell counts for the NSh-St group were: left MeA 87.7, BLA 29.7, CeA 16, LA 5.7 and right MeA 48.7, BLA 23.3, CeA 12.3, LA 5.7. * $p < 0.05$ left > right hemisphere; ** $p < 0.01$ vs corresponding no shock control within a region.

Figure 4. Freezing to conditioned cues. Percent freezing on the shock training day and during the 6 min test. Groups are shock-paired (Sh-P), shock-unpaired (Sh-U) and no shock (NoSh-P). ** $p < 0.01$ Sh-P and Sh-U vs NoSh-P on training and testing day; ++ $p < 0.01$ Sh-P vs Sh-U on the testing day.

Figure 5. Digitized c-Fos-stained sections with the amygdala regions outlined as in Figures 2A and 2A1. Sections were taken after the freezing test. A) Right hemisphere of a non shocked rat (NoSh-P). A1) Left hemisphere of a NoSh-P rat. B) Right hemisphere of a shocked rat tested in the no shock compartment (Sh-U). B1) Left hemisphere of a Sh-U rat. C) Right hemisphere of a shocked rat tested in the shock compartment (Sh-P). C1) Left hemisphere of a Sh-P rat.

Figure 6. Mean percent control of c-Fos positive cells (± SEM) in each amygdala region (abbreviations as in Fig 2) for the different behavioral groups (abbreviations as in Fig 4). The mean raw cell counts for the NSh-P group were: left MeA 68, BLA 15.8, CeA 11.3, LA 4.6 and right MeA 50.9, BLA 22.6, CeA 14.3, LA 5.3. ** $p < 0.01$ left > right hemisphere; ** $p < 0.01$ Sh-P vs Sh-U and NoSh-P within a region; ## $p < 0.01$ in left BLA Sh-U vs NoSh-P.

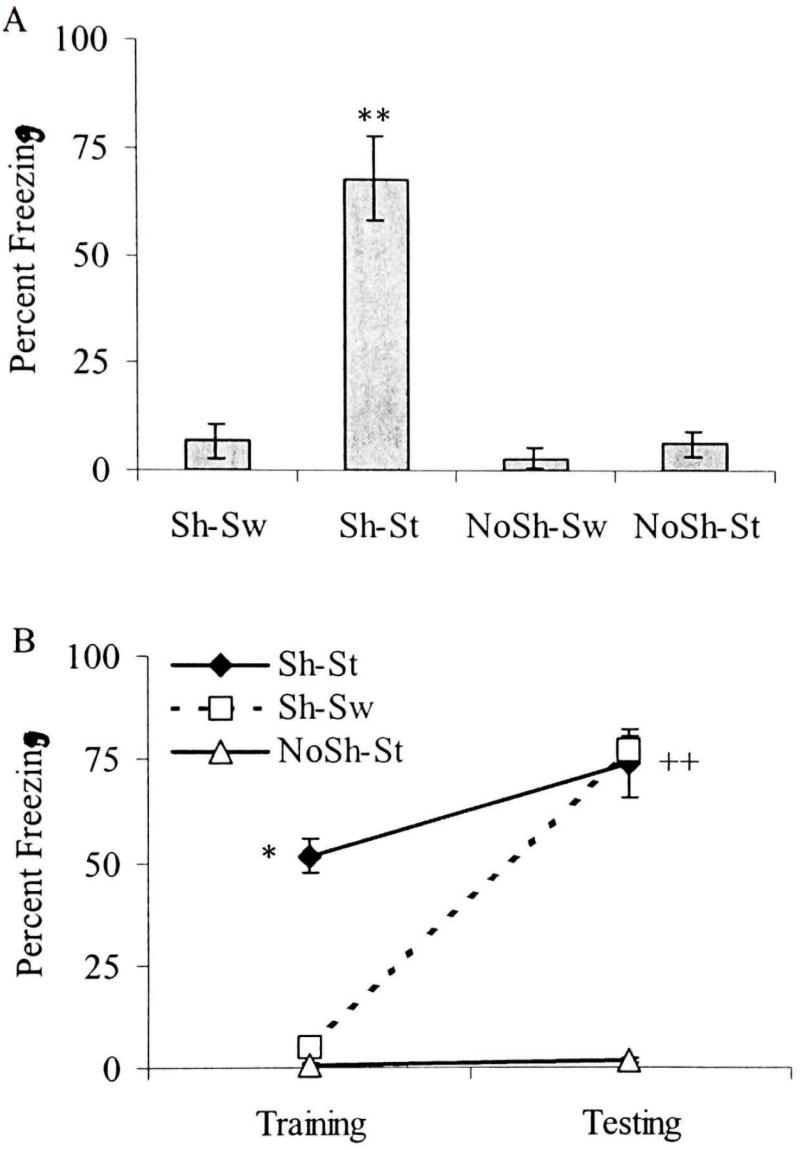


Figure 1, Holahan and White

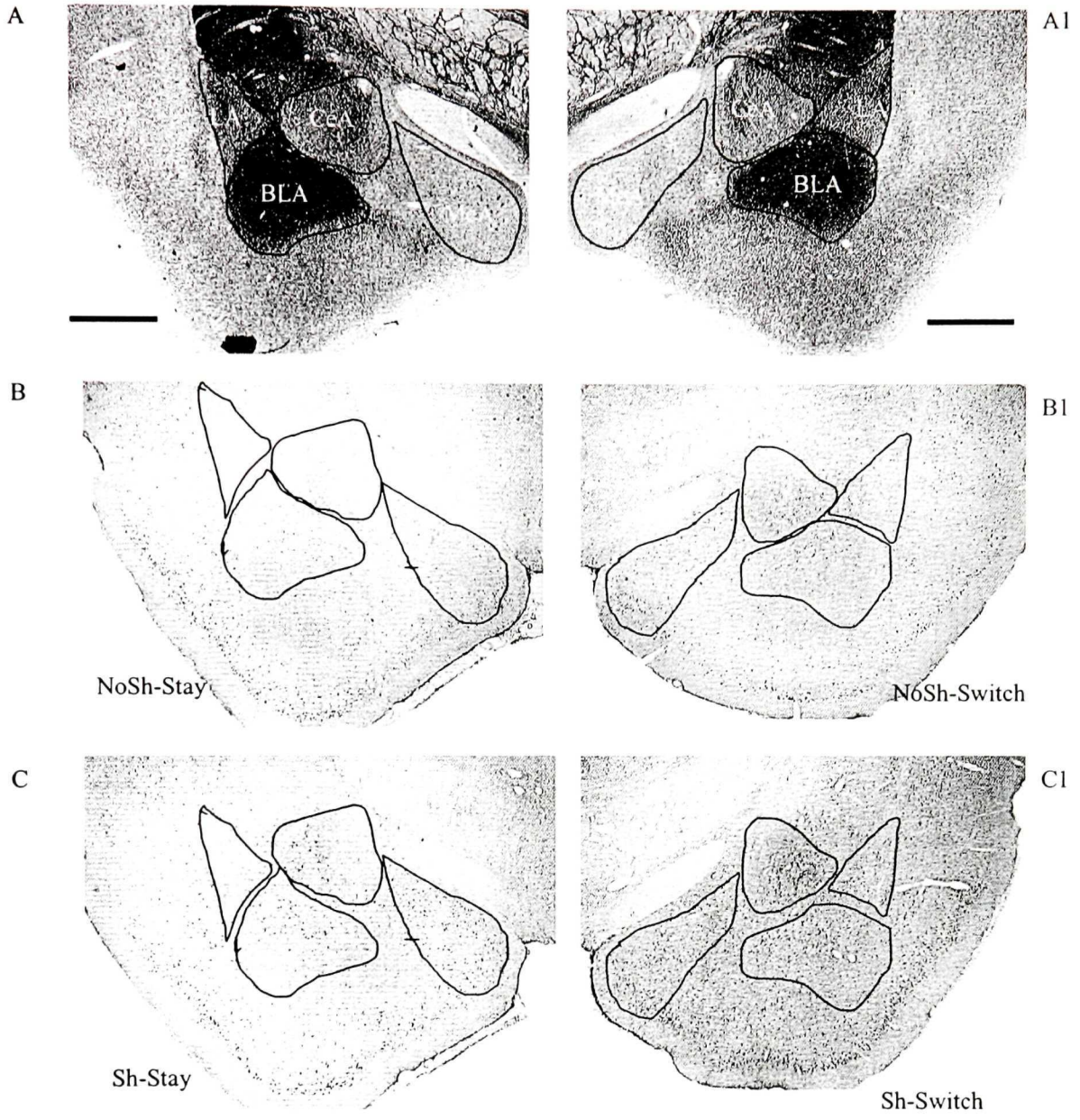


Figure 2, Holahan and White

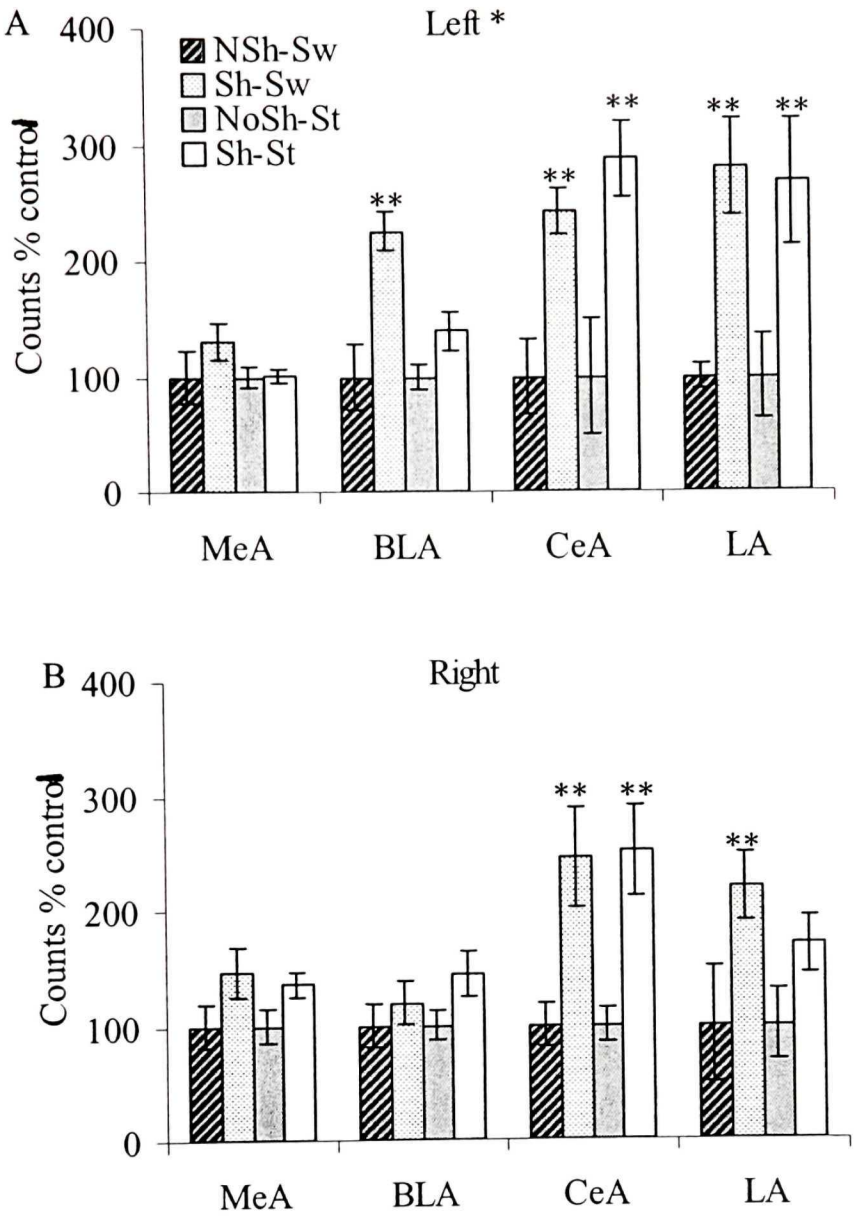
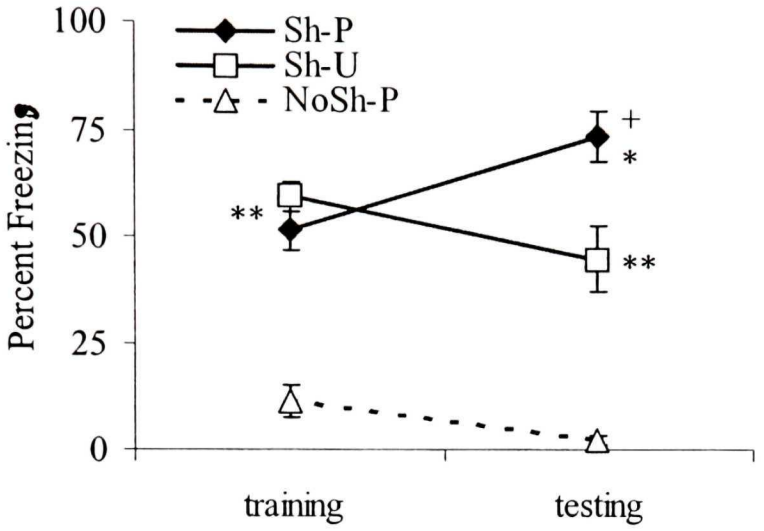


Figure 3, Holahan and White



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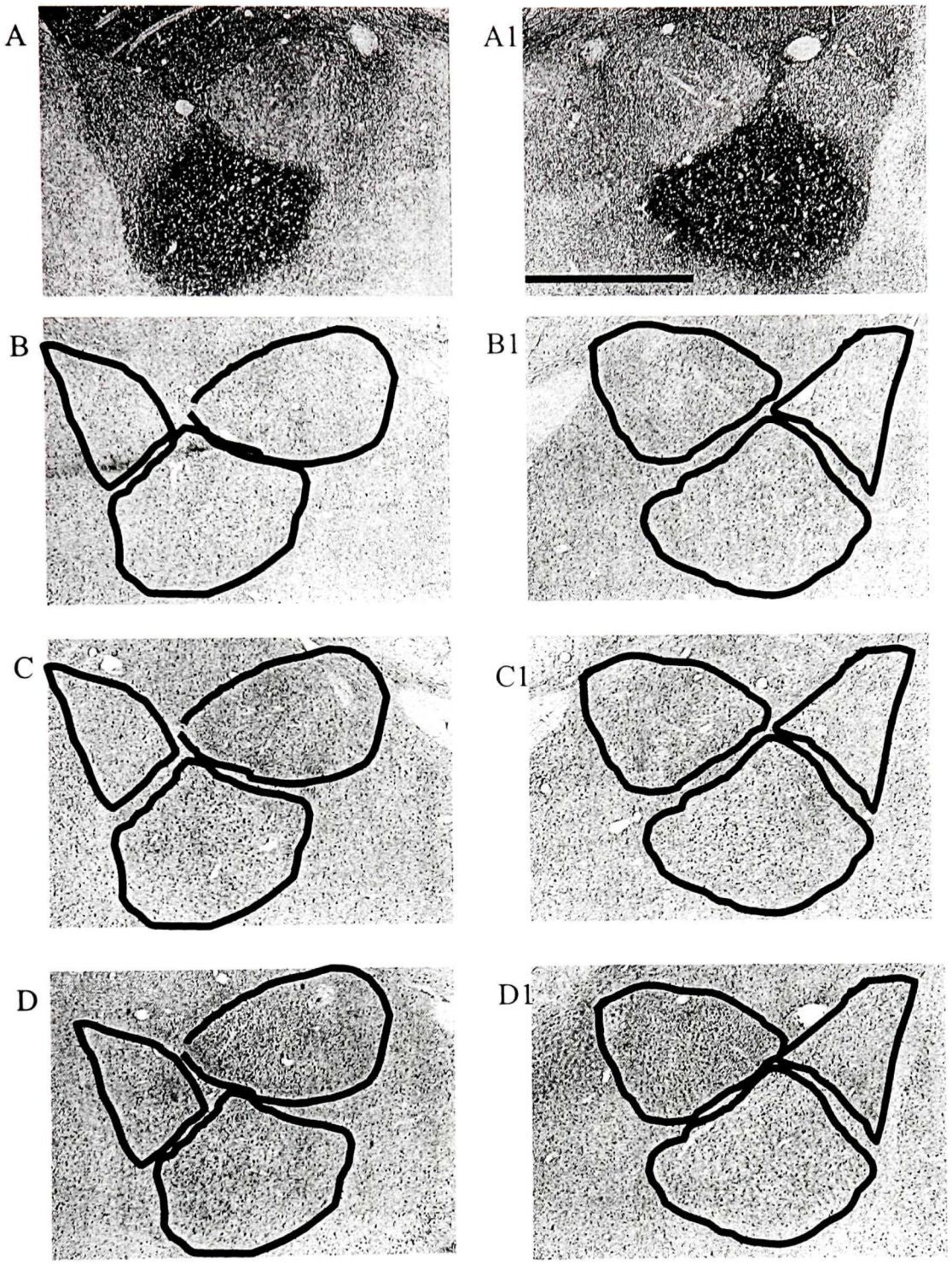

Figure 5, Holahan and White

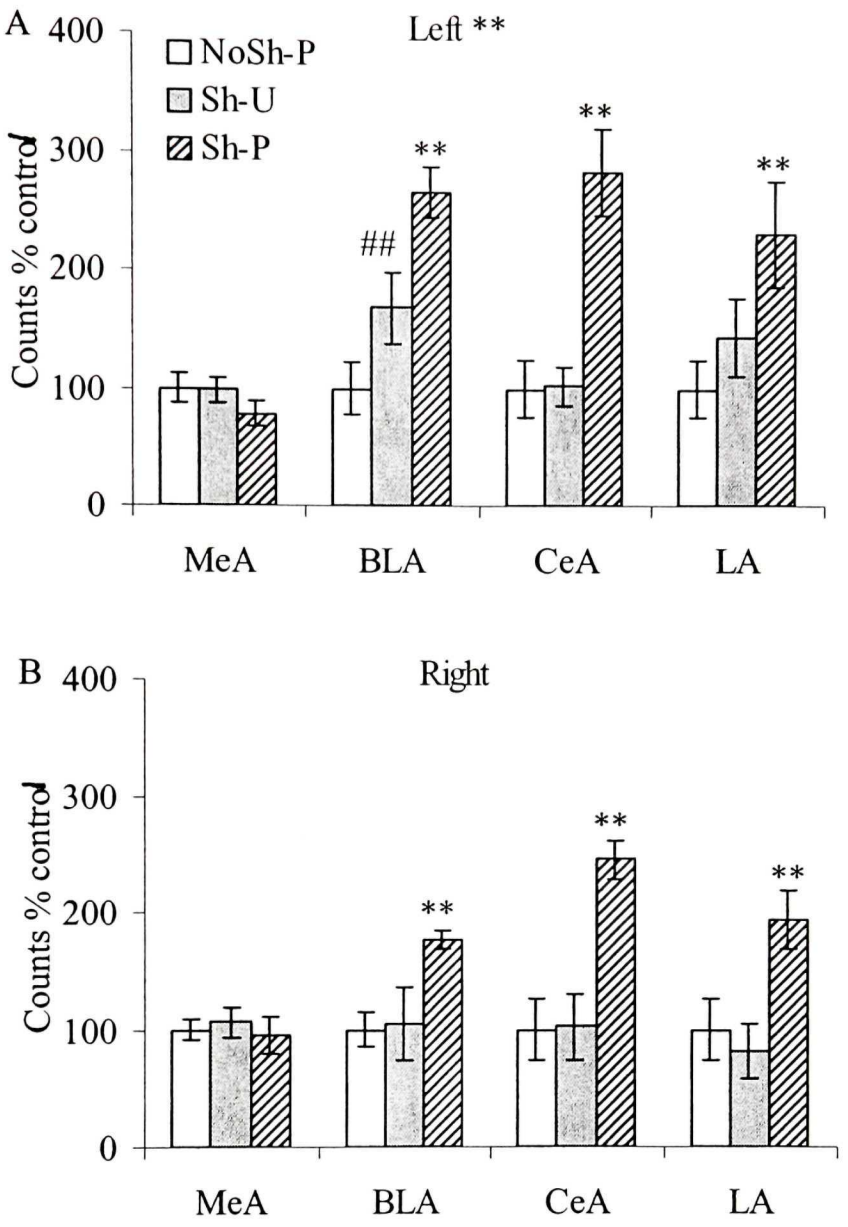

Figure 6, Holahan and White

Manuscript 2

In Manuscript 1, neither case of c-Fos expression was correlated with the occurrence of freezing. This activity may contribute to an aversive affective state (or "fear") and a variety of behaviors may be promoted by this state. Manuscript 2 examined this hypothesis by temporarily inactivating the amygdala during either training or testing for two incompatible behaviors (freezing and active place avoidance) in an aversive contextual conditioning procedure. The hypothesis that intra-amygdala muscimol injections impair the ability of rats to produce specific behaviors predicts that the rats' performance of either freezing (which requires inhibition of movement) or place avoidance (which requires initiation of movement) would be impaired. In contrast, the hypothesis that the amygdala mediates acquisition and expression of mnemonic information leading to an affective state that promotes both of these behaviors predicts that intra-amygdala muscimol injections would impair both behaviors.

Intra-amygdala muscimol injections impair acquisition and expression of incompatible behaviors in aversive contextual conditioning

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Running head: Amygdala and incompatible behaviors

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Key Words: conditioned fear, freezing, place avoidance, locomotor activity, amygdala, muscimol

Abstract

Although it is generally accepted that the amygdala is involved in aversive conditioning, it is unclear if manipulations of this structure affect an associative process or production of behaviors used to measure that process. This issue was examined by temporarily inactivating the amygdala during either training or testing for two incompatible behaviors in an aversive contextual conditioning procedure. Freezing was measured when the rats were confined in the presence of the aversive conditioned cues; place avoidance was measured when the same rats were allowed to move between the context containing the conditioned cues and a neutral context. Inactivation of the amygdala with pre-training injections of muscimol hydrobromide eliminated the increases in freezing produced by shock and attenuated freezing during subsequent exposure to the conditioned contextual cues. Avoidance of the conditioned context was eliminated. Pre-testing muscimol eliminated both behaviors. The muscimol injections did not reduce general activity. Since freezing requires cessation of movement and avoidance requires its initiation, these effects of muscimol cannot be attributed to a deficit in behavior production. Inactivation of the amygdala may have affected production of a conditioned aversive (affective) state that promotes either freezing or place avoidance, depending on environmental constraints, during exposure to the CS. Alternatively, amygdala inactivation may have affected a mnemonic process required for acquisition and expression of an association which produces this state.

Introduction

In aversive conditioning procedures, neutral cues such as those that comprise an experimental context (conditioned stimuli; CS) are paired with an aversive event such as footshock (unconditioned stimulus; US). Usually only a few CS-US pairings are required to produce a variety of behavioral changes that can be measured during exposure to the aversive CS (Davis, 1997; 2000; Fanselow, 1984; Fendt and Fanselow, 1999; LeDoux, 1998; 2000a).

Freezing, defined as a cessation of motor activity including whisker and nose movements (Bolles and Collier, 1976) or sitting rigidly motionless (Bindra and Anchel, 1963) except for movement necessitated by respiration (Fanselow, 1980), is usually increased in the presence of an aversive contextual CS (Blanchard and Blanchard, 1969; Bolles and Riley, 1973; Bouton and Bolles, 1980; Brown et al., 1951; Fanselow, 1984; LeDoux, 1996; 2000b; McAllister and McAllister, 1971).

Place avoidance can also be observed in the presence of an aversive contextual CS (Miller, 1948; Mowrer, 1947; Mowrer and Lamoreaux, 1946). In this case, rats that have been shocked in one compartment but not in another distinct compartment and then allowed to move freely between the two with no shock, spend more time in the neutral compartment than in the shock compartment (Antoniadis and McDonald, 1999; Blanchard and Blanchard, 1968; 1970b; 1970a; Campbell and Campbell, 1962; Goldstein, 1960; Holahan and White, 2002; Kumar, 1970; McAllister and McAllister, 1962; Miller, 1948; Selden et al., 1991; Vazdarjanova and McGaugh, 1998).

Although both behaviors occur in the presence of the same aversive CS, freezing and place avoidance are incompatible behaviors. Freezing is defined as the cessation of bodily movement but place avoidance requires that a rat move from one place to another. Clearly, the two behaviors cannot occur at the same time and expression of one interferes with expression of the other (Anisman, 1973; Anisman and Waller, 1972; 1973). The fact that they are both produced by the same experimental procedure suggests (but does not prove) the possibility that they may both result from some common underlying neural process.

The occurrence of these two behaviors depends to some extent on the configuration of the apparatus in which they are measured. Freezing occurs in the presence an aversive CS

when there is no opportunity to escape (Bindra and Anchel, 1963; Blanchard and Blanchard, 1969; Bolles and Collier, 1976; Fanselow, 1980; LeDoux, 1996). When there is some way to escape from exposure to the aversive CS (see for example Amorapanth et al., 2000; Blanchard and Blanchard, 1971; Holahan and White, 2002), place avoidance behaviors may emerge.

Amygdala lesions tend to block or attenuate the behavioral changes that result from aversive conditioning (Davis, 1997; Everitt et al., 2000; Fendt and Fanselow, 1999; LeDoux, 2000a). Freezing during exposure to a shock-paired compartment is attenuated by pre-training electrolytic lesions of the amygdala complex (Phillips and LeDoux, 1992), and lesions restricted to the medial (MeA) (Blanchard and Blanchard, 1972; Holahan and White, 2002), the central (CeA), or basolateral (BLA) (Holahan and White, 2002; Kim et al., 1993) amygdala regions. Pretraining (Cahill et al., 2000; Maren, 1998; 1999; Vazdarjanova and McGaugh, 1998) or post-training (Lee et al., 1996; Maren et al., 1996a; 1998) NMDA lesions of the BLA block freezing during exposure to a contextual CS. Pretraining NMDA lesions of the lateral amygdala (LA) or CeA (Goosens and Maren, 2001) also block freezing to a contextual CS.

Intra-amygdala injections of the NMDA antagonist AP5 reduce freezing during exposure to an aversive CS when given before training (Fanselow and Kim, 1994) or before testing (Fendt, 2001; Lee et al., 2001; Maren et al., 1996b). Lidocaine injected into the amygdala (Helmstetter, 1992) completely eliminated freezing when given before the test session but was less effective when administered prior to acquisition. The $GABA_A$ agonist muscimol disrupted freezing when injected into the BLA region before training or testing (Helmstetter and Bellgowan, 1994; Muller et al., 1997; Wilensky et al., 1999).

Place avoidance is also affected by amygdala lesions. Both electrolytic (Gaston and Freed, 1969) and NMDA (Antoniadis and McDonald, 2000) lesions of the entire amygdala impaired avoidance of a shock conditioned context.

Lesions restricted to specific amygdala regions have produced less consistent results. Neither electrolytic (Holahan and White, 2002), quinolinic (Selden et al., 1991), ibotenic (Ambrogi Lorenzini et al., 1991), nor NMDA (Vazdarjanova and McGaugh, 1998) lesions

of the BLA disrupted place avoidance. Electrolytic lesions of the CeA impaired avoidance of a shock-conditioned context (Holahan and White, 2002) but not avoidance of other aversive CSs (Amorapanth et al., 2000; Killcross et al., 1997).

Two general hypotheses have been proposed to explain deficits in freezing and place avoidance. It is possible that the amygdala functions as part of a circuit that stores information required for aversive CSs to produce changes in the level of freezing or avoidance (Fanselow and Gale, 2003; Fanselow and LeDoux, 1999; Fendt and Fanselow, 1999; LeDoux, 2000a; Maren, 1999; 2001). Alternatively, behavioral impairments produced by lesions or inactivation of the amygdala may result from impairments in the rats' ability to produce the behaviors (Cahill et al., 1999; 2000; Vazdarjanova et al., 2001).

To distinguish between these hypotheses, the present study examined the effects of temporary inactivation of the amygdala on the acquisition and expression of freezing and place avoidance. Intra-amygdala muscimol injections were given before rats were shocked in a context or before they were tested in the shock-paired context. Two separate tests were given: freezing was measured when the rats were confined in the shock-paired context; place avoidance was measured when the animals were allowed to move freely between the shock-paired context and an adjacent neutral context.

The hypothesis that intra-amygdala muscimol injections impair the ability of rats to produce specific behaviors predicts that the rats' performance of either freezing (which requires inhibition of movement) or place avoidance (which requires initiation of movement) would be impaired. In contrast, the hypothesis that the amygdala mediates acquisition and expression of mnemonic information leading to an affective state that promotes both of these behaviors predicts that intra-amygdala muscimol injections would impair both behaviors.

Results

Histology

Figure 1 shows representative sections of the brains of rats given pretraining or pretesting injections. The injector tips were located slightly dorsal to the CeA. No substantial damage to any part of the amygdala was produced by the injectors and there were no systematic differences among the groups in anterior-posterior placements.

C-Fos assessment

Figure 2 shows sections of the right and left hemispheres stained for c-Fos from representative rats in the test-mus and test-sal groups. The section from the rat injected with saline (test-sal) has more c-Fos positive cells ventral to the injector tip than the section from the rat injected with muscimol (test-mus). The section from the rat injected with muscimol has a zone of c-Fos suppression that included all amygdala regions (LA/BLA, CeA, and MeA) and the adjacent cortex ventral to the amygdala. It was not possible to determine whether the muscimol spread dorsally as both muscimol and saline injections produced dense c-Fos labeling around the cannula and injector tracks (Wang and Redgrave, 1997).

Figure 3 shows the means for each region analyzed in the left and right hemispheres. Analysis of these data revealed a main effect of group ($F(1,6) = 11.40$, $p < 0.02$) with no other main effects or interactions being significant. As seen in Figs 3a and 3b, intra-amygdala muscimol injections reduced c-Fos labeling in both the left and right LA/BLA, CeA, and MeA. This suggests that the dose and volume of muscimol was sufficient to reduce activity (as assessed by c-Fos labeling) in all amygdala regions. The data also indicate that normal activity was reduced in the adjacent cortical region ventral to the amygdala.

A previous report (Wang and Redgrave, 1997) found that 50 ng (0.44 nmol) of muscimol completely suppressed c-Fos expression in the superior colliculus while doses less than 50 ng produced incomplete c-Fos suppression. In that study, the zone of inactivation produced by an injection of 50 ng/ 0.5 μl dose of muscimol into the superior colliculus was approximately 0.5 mm (Wang and Redgrave, 1997). The 50 ng (0.44 nmol)/ 1.0 μl dose of muscimol used in the present study significantly suppressed c-Fos expression in the entire amygdala and spread at least 1.8 mm from the injector tip. This is consistent with previous measurements of the spread of radioactively labeled muscimol (Martin, 1991) injected at a dose of 1 μg/ 1.0 μl.

The dose of muscimol used in the present study has been shown to impair a variety of behaviors such as freezing (Helmstetter and Bellgowan, 1994; Muller et al., 1997; Wilensky et al., 1999; Wilensky et al., 2000), continuous multiple inhibitory avoidance (Coleman-Mesches and McGaugh, 1995), memory for magnitude of reward changes (Salinas and

McGaugh, 1996) and other types of instrumental behavior (Katoaka et al., 1987; Roberts et al., 1996). The present results suggest that these effects can be attributed to effects of muscimol in the amygdala, but do not rule out the possibility that suppression of neural activity in the adjacent cortex contributes to some or all of the effects of the injections. The results also preclude attributing effects to any specific amygdala subregion.

Closed door test

The I/F per second data during the closed door test are shown in Figure 4. Pre-training muscimol (Figure 4A) completely eliminated the increased I/F produced by footshock and attenuated the increased I/F produced by exposure to the conditioned cues during the test session. Pre-testing muscimol (Figure 4B) completely eliminated the increased I/F produced by exposure to the conditioned cues.

For the pretraining groups, there were significant main effects of group ($F(2,17) = 6.42$, $p < 0.01$) and phase ($F(2,4) = 55.94$, $p < 0.0001$) and a significant interaction ($F(4,34) = 10.39$, $p < 0.0001$). During the shock phase, group train-sal had a higher I/F score than groups train-mus and con-sal (t values > 4.2, $p < 0.01$) while the two latter groups did not differ ($t(12) = 1.16$). During the test, group train-sal had a higher mean I/F score than both groups con-sal ($t(12) = 4.71$, $p < 0.01$) and train-mus ($t(12) = 2.43$, $p < 0.05$), and group train-mus had a higher mean I/F score than group con-sal ($t(12) = 2.27$, $p < 0.05$). Group train-mus also showed a significant increase in I/F from the shock phase to the test phase ($t(12) = 4.20$, $p < 0.01$).

For the pretesting groups, there were significant main effects of group ($F(2,14) = 12.10$, $p < 0.001$) and phase ($F(2,4) = 39.90$, $p < 0.0001$) and a significant interaction ($F(4,28) = 12.67$, $p < 0.0001$). During the shock phase, I/F was lower in group con-sal than in groups test-sal and test-mus (t values > 5.5, $p < 0.01$). During the test phase, I/F in group test-sal was greater than in groups test-mus and con-sal (t values > 5.0, $p < 0.01$) but there was no significant difference between the two latter groups. During the test, mean I/F in group test-mus was not significantly different from its pre-shock level ($t(8) = 0.10$) but decreased significantly from the shock phase ($t(8) = 5.60$, $p < 0.01$).

Electrophysiological and autoradiographic studies show that an injection of 1 μg/ μl of

muscimol decreases spontaneous neural activity in brain tissue surrounding the injection site by 80% 2 min after the injection (Edeline et al., 2002) and that the maximum effect occurs 10 (Martin, 1991) to 25 (Edeline et al., 2002) min after the injections and lasts up to 2 hours (Edeline et al., 2002; Martin, 1991). These findings suggest that in the present study the drug was probably at maximum effectiveness in the pre-training group which was injected 40 min before training. This makes it unlikely that the partial attenuation of I/F during testing in this group was due to partial blockade of the amygdala during training. The data also suggest that the drug was highly effective in the pre-testing group which was injected 15 min before training. However, even if only a partial block occurred, it was sufficient to completely eliminate the elevated I/F produced by exposure to the conditioned cues.

Intra-amygdala muscimol injections during training could have produced a deficit in the ability to freeze (Cahill et al., 2000; Vazdarjanova et al., 2001). This hypothesis could explain the lack of an increase in I/F during training while the rats were under the influence of muscimol and, since such an effect would not necessarily affect the learning produced by the footshock, the absence of freezing during training is not necessarily inconsistent with the increase in I/F observed during the subsequent exposure to the conditioned cues. An alternative hypothesis is that amygdala inactivation could have blocked the modulation of information processed and stored in some other brain region(s) (Cahill et al., 1999; 2001; Packard et al., 1994; Packard and Cahill, 2001). In the absence of modulation, attenuated freezing such as that observed might be predicted.

The partial elimination of I/F during exposure to the conditioned cues is less consistent with the hypothesis that the amygdala is critical for mediating the storage of information required for behavioral changes produced by exposure to aversive cues (Fanselow and LeDoux, 1999; Maren, 2001; 2003). However, the observations could be explained by the hypothesis that information leading to freezing in these conditions is stored independently in more than one brain system (White and McDonald, 2002). According to this idea, output from each of these systems contributes to freezing during exposure to the CS. Elimination of one such output by inactivation of the amygdala eliminates its contribution but has no effect on the other output(s), which produce the attenuated freezing that was observed.

In the pretesting group, freezing produced by the CS was completely eliminated by intra-amygdala muscimol injections. Even if the amygdala was only partially inactivated under these conditions, this was sufficient to eliminate the behavior and shows that normal levels of neural activity within the amygdala are required for expression of freezing. In this case, muscimol could have impaired the ability to freeze (Cahill et al., 2000; Vazdarjanova et al., 2001) or impaired the retrieval and/or expression of a mnemonic representation (not necessarily stored in the amygdala) required to change the behavior (Fanselow and Gale, 2003; Fanselow and LeDoux, 1999; Maren, 2001; 2003)

Open door test

The avoidance data are shown in Figure 5. Both pre-training (Figure 5A) and pre-testing (Figure 5B) muscimol injections eliminated place avoidance behavior exhibited by the shocked, saline-injected groups.

For the pre-training groups, there was a significant group effect ($F(2,18) = 15.14$, $p < 0.001$) and significant differences between groups train-sal and con-sal ($t(12) = 5.16$, $p < 0.01$) and between groups train-sal and train-mus ($t(12) = 4.23$, $p < 0.01$). The avoidance ratios for groups con-sal and train-mus were not significantly different ($t(12) = 0.93$).

For the pre-testing groups, there was a significant group effect ($F(2,17) = 13.64$, $p < 0.001$) and significant differences between groups test-sal and con-sal ($t(11) = 5.03$, $p < 0.01$) and between groups test-sal and test-mus ($t(12) = 3.66$, $p < 0.01$). The avoidance ratios for groups con-sal and test-mus ($t(11) = 1.51$) were not significantly different.

In the pretraining groups, the rats were tested first with the door closed and then with the door open, which may have led to some extinction during the closed door test. If this occurred, elimination of the place avoidance behavior in the muscimol group might have been due to the fact that the rats were tested with a partially extinguished conditioned affective state. Two results argue against this interpretation. First, observations of freezing during the first bout in the paired compartment of the open door test (see Table 1) show that more rats froze in the train-sal than the test-sal condition. Second, the avoidance ratio was 0.62 in the train-sal group and 0.57 in the test-sal group. Neither of these comparisons is consistent with the extinction hypothesis.

The large avoidance ratios for the shocked, saline-injected groups are consistent with the hypothesis discussed in the introduction concerning this form of avoidance behavior, which is thought to be due to acquisition of a conditioned aversive affective state during training and learning new instrumental behaviors that eliminate the conditioned aversive state during testing (Miller, 1948; Mowrer, 1947; Mowrer and Lamoreaux, 1946).

In this experiment, pre-training muscimol must have impaired acquisition of the hypothetical conditioned aversive state during the training session. Pre-testing muscimol could have acted in one or more of several ways. It could have impaired the ability to initiate the avoidance behavior, impaired retrieval of the conditioned internal response, or it could have impaired acquisition of the instrumental behavior.

Crossovers

The crossover rates are shown in Figure 6. For the groups injected before training (Figure 6a) there was a significant group effect ($F(2,18) = 4.89$, $p < 0.05$) and significant differences between groups train-sal and con-sal ($t(12) = 3.48$, $p < 0.01$) and between groups train-sal and train-mus ($t(12) = 2.08$, $p < 0.05$). The crossover rates for groups con-sal and train-mus were not significantly different ($t(12) = 0.76$).

For the groups injected before testing (Figure 6a) there was a significant group effect ($F(2,17) = 21.97$, $p < 0.001$) and significant differences between groups test-sal and con-sal ($t(11) = 2.82$, $p < 0.01$) and between groups test-sal and test-mus ($t(12) = 6.55$, $p < 0.01$). The crossover rates for groups con-sal and test-mus were also significantly different ($t(11) = 3.47$, $p < 0.01$).

Crossover rates, which involve moving between the two compartments can be taken as a measure of general activity. Among the experimental groups that received injections of saline, crossovers were lower in the groups that were shocked during the training phase than in the groups that were not shocked. This is consistent with findings showing that exposure to shock-conditioned cues reduces activity levels (Blanchard and Blanchard, 1969; Brener and Goesling, 1970; Kumar, 1970).

Intra-amygdala muscimol injections given before the shock phase resulted in crossover rates that were similar to the non-shocked group and higher than the shock-saline group. In

the shocked group injected with muscimol before the test, the crossover rate was higher than both the shocked and non-shocked groups injected with saline.

The elevated number of crossovers during the open door test suggests that muscimol inactivation of the amygdala produced an increase in locomotor activity and the possibility that this interfered with the expression of freezing behavior. While it has been reported by some that amygdala lesions do not produce changes in activity (Decker et al., 1995; Fanselow and Gale, 2003; Goldstein, 1968; Maren, 1998) the present results showed that muscimol injections increased activity. Previous findings found that lesions of the BLA (Holahan and White, 2002) or muscimol injections into the BLA (Holahan and White, 2003) increased locomotor activity. An increase in activity may have overshadowed the ability to detect elevations in freezing when the rats were under the influence of muscimol during closed door training and testing. This would confound any interpretation of a blockade of associative information based on the freezing data.

An alternative hypothesis would be that the increase in activity was due to a reduction in an affective "fearful" state experienced during exposure to the aversive CS and the new apparatus configuration. As the rats experienced the open door configuration for the first time during the place avoidance test, this would constitute a novel environment. When normal rats are first placed into a novel environment they may experience fear and have a tendency to freeze (Baron, 1964; Zangrossi and File, 1992). With increased exposure they begin to explore (Suess and Berlyne, 1978). Depending on the complexity of the environment, the measures of exploration (locomotion) can be quite high (Barnett, 1963; Suess and Berlyne, 1978). As the environment becomes familiar, exploration decreases to a very low level. This pattern leads to an inverted U-shaped function of activity over time. This pattern has been attributed to changes in levels of fear: high levels produce freezing (Blanchard and Blanchard, 1969; Brener and Goesling, 1970; Kumar, 1970), while slightly lower levels produce exploration (Barnett, 1963), and lowest levels produce low levels of exploration (sleep). On this view, the increase in activity levels produced by muscimol in the present experiment would be due to a decrease in conditioned fear from the highest to moderate levels.

Discussion

Intra-amygdala muscimol injections completely eliminated the freezing observed in normal rats during shock. When exposed to the shock-paired cues, rats injected with muscimol during training froze less, failed to avoid the shock-paired cues, and were more active than shocked controls with normal amygdala function during training. Rats that were trained in a normal state and given intra-amygdala muscimol injections before testing froze less, failed to avoid the shock-paired cues, and were more active than shocked controls trained and tested in the normal state.

These results have implications for 4 general hypotheses concerning amygdala function: 1) the amygdala is required for the production of overt behaviors such as freezing and place avoidance; 2) the amygdala mediates the production of a set of covert responses, including hormonal, autonomic and neural activity, that constitute an aversive affective state; 3) the amygdala modulates memories stored in other brain regions; 4) the amygdala is a critical part of a neural system that stores representations of the elements of Pavlovian-type associations. The implications of the present results for each of these hypotheses will be discussed in turn.

Overt Behaviors

Reductions in freezing produced by lesions or inactivation of the amygdala may result from impairments in the rats' ability to produce the behavior (Cahill et al., 1999; 2000; Vazdarjanova et al., 2001). In the present study intra-amygdala muscimol injections eliminated freezing during the 4 minute shock phase and the 6 min test. This is consistent with previous reports (Helmstetter and Bellgowan, 1994; Muller et al., 1997; Wilensky et al., 1999; 2000). However, as argued by some (Bolles, 1970) increased activity (i.e., lack of freezing) in the standard test for freezing could be due to an intact (or even enhanced) tendency to actively avoid the aversive CS. This would suggest that a mnemonic representation remained intact and the ability to freeze was compromised.

In the present study, both freezing and place avoidance were measured during exposure to the CS. As freezing tends to interfere with the expression of active, avoidance behaviors (Anisman, 1973; Anisman and Waller, 1972; 1973), the measurement of these incompatible behaviors can control for the possibility that the intra-amygdala muscimol injections affected

the ability to inhibit or initiate specific overt behaviors. If the rats' ability to freeze had been affected, they should still have exhibited place avoidance. Similarly, if their ability to perform the avoidance behavior had been affected, they should still have frozen. The fact that both of these behaviors were reduced while the rats remained normally active suggests that the injections did not affect the rats' ability to perform either of these behaviors. These opposing effects are inconsistent with the hypothesis that the amygdala is required for the production of specific overt behaviors and, consequently, that the effects of amygdala inactivation or lesions are due to impairments in the ability of rats to perform these behaviors.

Aversive Affective State

An aversive affective state, commonly referred to as fear, is often used as an intervening variable to explain various behavioral changes produced during aversive conditioning (Brown and Jacobs, 1949; Davis, 1997; Fanselow, 1984; LeDoux, 2000a; McAllister and McAllister, 1995; Miller, 1948; Mowrer, 1947; Rescorla and Solomon, 1967). Affective states are hypothesized to be comprised from arrays of physiological responses, and there is evidence that these responses can participate in the promotion of overt behaviors. Chemical or electrical stimulation of the amygdala (Iwata et al., 1987; Kapp et al., 1982; Sanders and Shekhar, 1991) elevates heart rate and blood pressure. Sanders and Shekhar (1991) also reported that intra-amygdala injections of a $GABA_A$ *antagonist* (bicuculline) elevated heart rate and blood pressure, and similar injections have been shown to produce conditioned place avoidance (Thielen and Shekhar, 2002). These physiological responses may contribute to an aversive affective state that promotes overt behaviors. Activation of the amygdala GABA system, as in the present study, would block the production of this state, reducing the occurrence of avoidance and, possibly of other overt behaviors.

The present findings are consistent with the hypothesis that a normally functioning amygdala mediates a conditioned aversive affective state. Accordingly, the suggestion that the present effects of amygdala inactivation on freezing and avoidance were due to blocking the responses that constitute an affective state is a viable hypothesis. Moreover, in contrast to the hypothesis that amygdala lesions block overt behaviors, the hypothesis that they block internal responses can explain the effects of amygdala inactivation in the present experiment,

and the effects of inactivations and lesions in other experiments (for review see Davis, 1997; LeDoux, 2000a; Maren, 2001).

Memory Modulation

There is good evidence that output from the amygdala modulates (i.e., strengthens) memories, including those stored in other brain areas (McGaugh et al., 1996; 2000; Packard and Cahill, 2001; Packard and Teather, 1998). It has been suggested (Holahan and White, 2002; White and McDonald, 2002) that a subset of the physiological responses that comprise the aversive internal state produce this effect. In the present experiment, inactivation of the amygdala during the training trials may have blocked these responses (see also Salinas and McGaugh, 1996), resulting in a failure to store the association required to produce the conditioned responses.

The hippocampus is one other brain region that has been implicated in the learning that occurs during aversive conditioning. Disruptions in both conditioned contextual freezing (Antoniadis and McDonald, 2000; Kim et al., 1993; Kim and Fanselow, 1992; Maren et al., 1997; McNish et al., 1997; Phillips and LeDoux, 1992) and place avoidance (Antoniadis and McDonald, 2000; Cimadevilla et al., 2000; Isaacson et al., 1961; Selden et al., 1991) have been reported following hippocampus lesions. Hippocampus-based memories are modulated by the amygdala (Packard and Teather, 1998). Accordingly, in the absence of amygdala-based modulation during training in the present study, less information may have been stored in the hippocampus, resulting in attenuated behavioral output.

A second brain region involved in information processing during aversive conditioning is the dorsal striatum. Lesions of the dorsal striatum block active avoidance behaviors (Kirkby and Kimble, 1968; Kirkby and Polgar, 1974), suppression (Viaud and White, 1989), and freezing (Salinas and White, 1997) in the presence of shock-paired cues. As in the case of the hippocampus, impaired amygdala modulation of information storage in the dorsal striatum (Packard and Teather, 1998) could account for the present behavioral deficits.

The memory modulation hypothesis of amygdala function can account for the behavioral deficits produced by pretraining intra-amygdala muscimol injections. However, this hypothesis cannot account for the disruption of both freezing and place avoidance with

pretesting intra-amygdala muscimol injections.

Memory Storage

According to one recent theory (White and McDonald, 2002), memories are stored in neural systems; damage to major components of these systems impairs their mnemonic functions. Much of the evidence on aversive conditioning is consistent with the idea that the amygdala is a critical part of a system that mediates memories of the Pavlovian type (Fanselow and Gale, 2003; Fanselow and LeDoux, 1999; Maren, 2001; 2003), in which the conditioned response is an aversive affective state.

The memory storage hypothesis suggests that the behavioral deficits observed in the present experiment could be due to a disruption of mnemonic information mediated by the amygdala memory system. In one version of this hypothesis the amygdala mediates a representation of some part of the CS-US association during aversive conditioning (cf. Fanselow and Gale, 2003; Kesner, 1998; Kesner and Gilbert, 2001). In the present set of experiments, pretraining muscimol would have blocked the acquisition of this representation and pretesting muscimol would have blocked its retrieval. Alterations in amygdala neurons are hypothesized to be the basis of this stored information (Fanselow and LeDoux, 1999).

Neither the present findings, nor others involving behavioral observation permit a distinction between the hypotheses that the amygdala is the substrate of the memory that produces a conditioned internal state or that it simply produces the state. Possibly the issue is moot. If a representation of the CS-US association is mediated in the amygdala, and if the amygdala produces the internal state - that is, the conditioned response - the amygdala contains all the elements of a Pavlovian association. In this case, there is no distinction between eliminating the memory and eliminating the response it produces.

The dorsal striatum and hippocampus may be parts of other neural systems that mediate different kinds of learning during aversive conditioning (White and McDonald, 2002), suggesting that behaviors observed during aversive conditioning may not always be produced solely by amygdala-mediated Pavlovian conditioning. Such behaviors could include various dorsal striatum - mediated stimulus-response associations and the hippocampus-mediated perception of an impending aversive event (or outcome). Further research will be required

to examine the contributions of all of these processes to observed aversive behaviors.

Summary

Observations of the effects of amygdala inactivation during training or testing on two incompatible behaviors acquired during aversive conditioning, freezing and place avoidance, have implications for several hypotheses of aversive learning. In general, the findings do not support the hypothesis that the observed behaviors are produced directly by amygdala output, and they limit the hypothesis that they are produced by amygdala-based memory modulation. The present findings do not refute either the ideas that the amygdala mediates the production of conditioned aversive states or that the amygdala is part of a system that stores the associations that produce these states. Accordingly, the amygdala may mediate acquisition of a mnemonic representation during aversive conditioning, activation of which produces a conditioned aversive affective state. This affective state may promote the expression of overt behaviors depending on the environmental configuration.

Lesions or inactivation of the amygdala would block this process, eliminating its contribution to the overt behaviors that occur during exposure to conditioned cues. Output from other memory systems may continue to produce overt behaviors, some or all of which may be similar to those produced by the amygdala.

Materials and Methods

Subjects

Male, Long-Evans rats (n = 58) from Charles River (St. Constant, Québec, Canada) weighed 250 - 300 g at the beginning of the experiment. They were singly housed in hanging wire cages with water freely available in a temperature (21° C ± 2°) and light (on 700h: off 1900) controlled room. Rats were treated in accordance with guidelines of the Canadian Council on Animal Care and protocols approved by the McGill University Animal Care and Use Committee.

Surgery

Twenty-four hour food-deprived rats were anaesthetized with an intraperitoneal injection of 65 mg/kg sodium pentobarbital, given 5 mg/kg atropine sulphate subcutaneously, and underwent standard, stereotaxic surgery with the tooth bar set at - 3.5 mm (Paxinos and

Watson, 1998). Guide cannulas (26 ga, 11 mm length) were implanted at coordinates AP - 2.5, ML ± 4.2, DV - 6.0 in mm from bregma and the skull surface. Each rat was then given an intramuscular injection of penicillin (300,000 units/ ml) and placed into a heated holding cage. When recovery from anesthesia began (gross motor movements observed) each rat was given 0.01 ml Dipyrone (subcutaneous) to relieve post-surgical discomfort.

Apparatus

The shuttle-box consisted of two adjacent stainless steel compartments (29 x 28 x 24 cm) resting on two stainless steel catch pans 6 cm below the floor. The front walls were clear Plexiglas, the walls of one compartment were grey, and the walls of the other were black and white checkered. There was a connecting guillotine-type door in the center of the common wall. The floors of each compartment consisted of 0.5 cm diameter stainless steel rods spaced 1.5 cm apart connected to shock generators. It was previously reported (Holahan and White, 2002) that rats do not show any spontaneous unconditioned side preferences in this apparatus.

A passive infra-red motion detector (Radio Shack, 49-550) modified to be optimally sensitive to the infra-red wavelength emitted by rodents (R.E. Brown, personal communication) was mounted over a 6 cm diameter hole in the top of each compartment. The detector responded to whole body movements, but not to small movements confined to the head or tail or to sniffing. The output of the detector was sampled 20 times per s by a computer. If a movement was in progress at the time a sample was taken, a count was recorded. The counts were accumulated in 20 s bins; the maximum count for each bin was 400. An inactivity/freezing (I/F) score was calculated for each 20 s bin by subtracting the number of counts accumulated in the bin from 400. The I/F score reflected a combination of the standard freezing measure (Bolles and Collier, 1976; Fanselow, 1980) and a certain amount of whole body inactivity that might not be included in the definition used by others. However, I/F and freezing are highly correlated (Holahan and White, 2002). The detectors were also used to determine the amount of time the rats spent in each compartment and the number of times they moved between the compartments (crossovers) during open door testing.

A third box (Box C; 29 x 28 x 24 cm) was also used. This box was in the same room as

the shuttle-box but was not attached to it. The frame of Box C was constructed from wood, the walls and ceiling were 1.0 cm wire mesh, and the floor was a metal sheet.

Handling

The rats were acclimatized to the animal housing conditions for one week, during which they were handled for 4 consecutive days in the animal housing room. Eight to ten rats were placed into a large plastic tub with wood chips covering the floor for 2 h. Each rat was held by the experimenter for 5 min per day. On the third handling day, each rat was given an intra-amygdala injection of saline. The stylets were removed and replaced with 32 ga injector cannulas connected via plastic tubing to a minipump. The injectors extended 1.5 mm beyond the guide cannulas. The experimenter held the rat while 1.0 μl of saline was injected over 3 min. After the injection, the stylets were replaced and the rats were put back into their home cages. Rats were fed 30 - 35 g of food after the end of each day of the procedure at 1600; approximately 4 h after the end of each day's testing.

Pre-exposure

On each of the two days following handling each rat was confined in Box C for 15 min. On the following day each rat was randomly assigned to paired and unpaired compartments in the shuttle-box. When a compartment was paired, the rod floor was exposed; when it was unpaired, the rod floor was covered with a 0.5 cm wire mesh. The designation of the two compartments as paired and unpaired was counterbalanced within each experimental group. Each rat was confined in its paired compartment for 6 min and then immediately moved by the experimenter to its unpaired compartment for 6 min. During pre-exposure, the connecting door between the compartments was closed and no shocks were given.

Pretraining injections

Twenty four h after pre-exposure to the shuttle-box, 21 rats were given intra-amygdala saline or muscimol injections using the procedure described under *Handling*. Seven rats were injected with muscimol hydrobromide (Sigma; 0.44 nmol free base weight/ 1.0 μl; 1 μl per side over 3 min) and 14 rats were injected with the same volume of saline. The injectors were left in place for 2 min following each injection.

Forty min after the injections, 7 muscimol (train-mus) and 7 saline (train-sal) rats were

placed into their paired compartments. After 2 min (pre-shock phase) they were given four 0.5 s, 1.0 mA foot shocks (shock phase) with a 1 min inter-shock interval. The 7 remaining saline-injected rats (con-sal) were placed into their paired compartments but were not given any shocks. The next day, all rats were placed into their unpaired compartments for 6 min with no injections and no shocks.

Twenty four h after exposure to their unpaired compartments, each rat was placed into the shock-paired compartment with the connecting door between the compartments closed (test phase). The rats remained there for 6 min and the detector recorded I/F.

Twenty-four h after the closed door test, all rats were placed into the shock-paired compartment with the connecting door between the compartments open. During the 12 min test, time in each compartment and the number of times the rats moved into and out of the paired compartment (crossovers) were recorded automatically by the detectors.

Pretesting injections

Twenty-five rats were placed into their paired compartments and shocked as described for the pre-training groups. The remaining 12 rats were placed into their paired compartments but were not given any shocks. The next day, all rats were placed into their unpaired compartments for 6 min with no shocks.

Twenty four hours after exposure to their unpaired compartments, shocked rats were injected with muscimol (test-mus; n = 12) or saline (test-sal; n = 13). The 12 non-shocked rats (con-sal) were injected with saline.

Fifteen min following the injections, 5 rats in group test-mus and 6 rats each in groups test-sal and con-sal were placed into their shock-paired compartments for 6 min with the connecting door between the compartments closed. I/F was recorded using the detectors.

The remaining 7 rats in groups test-mus and test-sal and 6 rats in group con-sal were placed into the shock-paired compartment with the connecting door between the compartments open. Time in each compartment and crossovers between the compartments were recorded automatically during the 12 min test.

Data Analysis

For the closed door test, I/F during the 2 min (6 20 s bins = 120 s) before rats received

shock in their paired compartments was labeled the "pre-shock" phase. I/F during the 4 min (12 20 s bins = 240 sec) when the rats received the 4 shocks was the "shock phase". The total I/F for each phase was divided by the duration of the phase to obtain an I/F per s score. Similarly, total I/F for the 18 bins that comprised the 6 min closed door test phase was summed and divided by 360. The I/F per s scores for the three phases were analyzed with a two way repeated measures ANOVA (group by phase) and Fisher's least significant difference (LSD) post hoc tests (Maxwell and Delaney, 1990).

The place avoidance test was the first time the rats were exposed to the open door configuration. In most normal, untreated rats, exposure to a novel environment elicits exploration (Barnett, 1963). In the present case this led to discovery of the open door and escape from the shock-paired context. In some shocked rats injected with saline (see Table 1), freezing and other forms of inactivity observed only during the first bout in the paired compartment impeded this behavior (Anisman, 1973; Anisman and Waller, 1973; Baron, 1964; Brener and Goesling, 1970; Kumar, 1970; Sidman, 1962). This increased the duration of the first bout in the paired compartment for these rats (Table 1), distorting the place avoidance measure. To obtain an uncontaminated measure of place avoidance, the data for this bout were removed from the total time in the paired compartment for all rats. Avoidance ratios were calculated as the total amount of time spent in the unpaired compartment minus the amount of time in the paired compartment (without the first paired bout) divided by the sum of these two times [(unpaired - paired)/ (unpaired + paired)]. The avoidance ratios were analyzed with a randomized one way ANOVA and Fisher's LSD post hoc tests.

Movement between the two compartments was expressed as a crossover rate, defined as the total number of times a rat moved between the paired and unpaired compartments divided by the session time remaining after subtraction of the duration of the first bout in the paired compartment. In these calculations, the number of crossovers was reduced by 1 to correct for elimination of the first bout, which was ended by the first crossover. Crossover rates were analyzed with a randomized one way ANOVA and Fisher's LSD post hoc tests.

Histology

Upon completion of the behavioral procedures, rats were overdosed with an

intraperitoneal injection of 60 mg/kg chloral hydrate and perfused transcardially with saline followed by 10% formal-saline. Brains were post-fixed for approximately one week in formal-saline before being frozen and cut through the implant tracks at 30 μm. Brain slices were mounted on gelatin coated slides, allowed to air dry for 2 days, and stained with thionin (Donovick, 1974). Whole brain slices were digitally captured using Scion Image and processed with CorelDraw 10.

Assessment of c-Fos expression

To examine the diffusion of muscimol injected into the amygdala, a subset of the rats given pretesting injections (n = 4 test-sal and 4 test-mus) were sacrificed 90 min after the end of the closed door test and their brains were processed for c-Fos protein labeling using a procedure similar to that supplied by Oncogene Research Products (Protocol 1: Staining *fos* induced formalin-fixed, floating rat brain sections with *c-fos* (Ab-5) courtesy of J. Elmquist and C.B. Saper). The primary antibody (polyclonal c-Fos Ab-5; 1: 50,000; lot # D09803) was from Calbiochem. The secondary antibody (biotinylated anti-rabbit made in goat) and the avidin-biotin complex (ABC Elite Kit) were from Vector.

During slicing, every second brain section through the cannulae tracks from each rat was saved for thionin staining. This stain produced visible differentiation of the lateral/basolateral amygdala (LA/BLA), the central amygdala (CeA), and the medial amygdala (MeA). The cells in the piriform cortex and the cortical amygdala were also visible. An image of a brain section just caudal to the injector track was captured and enlarged 4x using a microscope and imaging software. The region of interest was outlined on these images and transferred to the adjacent section, which had been processed for c-Fos. Labeled cells within these regions were counted by an experimenter without knowledge of the experimental group to which the brain sections belonged.

For each rat in the test-sal and test-mus groups the c-Fos positive cells for the 4 regions in the left and right hemispheres were calculated as percentages of the mean counts for the test-sal group. This gave a mean of 100% for each region in the test-sal group and a variance around that mean. It also gave means and variances for each region in the test-mus group that could be compared to the means of the test-sal group using a 3-way ANOVA with group

(muscimol or saline) as the between factor and hemisphere (left or right) and region of interest (LA/BLA, CeA, MeA, cortical area) as repeated measures.

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References

Ambrogi Lorenzini,C., Bucherelli,C., Giachetti,A., Mugnai,L., and Tassoni,G. 1991. Effects of nucleus basolateralis amygdalae neurotoxic lesions on aversive conditioning in the rat. Physiol. Behav. 49:765-770.

Amorapanth,P., LeDoux,J.E., and Nader,K. 2000. Different lateral amygdala outputs mediate reactions and actions elicited by a fear-arousing stimulus. Nat. Neurosci. 3:74-79.

Anisman,H. 1973. Effects of pretraining compatible and incompatible responses on subsequent one-way and shuttle-avoidance performance in rats. J. Comp. Physiol. Psychol. 82:95-104.

Anisman,H. and Waller,T.G. 1972. Facilitative and disruptive effects of prior exposure to shock on subsequent avoidance performance. J. Comp. Physiol. Psychol. 78:113-122.

Anisman,H. and Waller,T.G. 1973. Effects of inescapable shock on subsequent avoidance performance: role of response repertoire changes. Behav. Bio. 9:331-355.

Antoniadis,E.A. and McDonald,R.J. 1999. Discriminative fear conditioning to context expressed by multiple measures of fear in the rat. Behav. Brain Res. 101:1-13.

Antoniadis,E.A. and McDonald,R.J. 2000. Amygdala, hippocampus and discriminative fear conditioning to context. Behav. Brain Res. 108:1-19.

Barnett,S.A. 1963. Movement in the living space. In the rat: A study in behavior, pp. 15-33. Aldine Publishing Company, Chicago, IL.

Baron,A. 1964. Suppression of exploratory behavior by aversive stimulation. J. Comp. Physiol. Psychol. 57:299-301.

Bindra,D. and Anchel,H. 1963. Immobility as an avoidance response, and its disruption by drugs. J. Exp. Anal. Behav. 6:213-218.

Blanchard,D.C. and Blanchard,R.J. 1972. Innate and conditioned reactions to threat in rats with amygdaloid lesions. J. Comp. Physiol. Psychol. 81:281-290.

Blanchard,R.J. and Blanchard,C. 1968. Escape and avoidance responses to a fear eliciting situation. Psychon. Sci. 13:19-20.

Blanchard,R.J. and Blanchard,C. 1969. Crouching as an index of fear. J. Comp. Physiol. Psychol. 67:370-375.

Blanchard,R.J. and Blanchard,D.C. 1970a. Dual mechanisms in passive avoidance: I. Psychon. Sci. 19:1-2.

Blanchard,R.J. and Blanchard,D.C. 1970b. Dual mechanisms in passive avoidance: II. Psychon. Sci. 19:3-4.

Blanchard,R.J. and Blanchard,D.C. 1971. Defensive reactions in the albino rat. Learn. Mot. 2:351-362.

Bolles,R.C. and Collier,A.C. 1976. The effect of predictive cues on freezing in rats. Anim. Learn. Behav. 4:6-8.

Bolles,R.C. 1970. Species-specific defensive reactions and avoidance learning. Psychol. Rev. 77:32-48.

Bolles,R.C. and Riley,A.L. 1973. Freezing as an avoidance response: another look at the operant-respondent distinction. Learn. Mot. 4:268-275.

Bouton,M.E. and Bolles,R.C. 1980. Conditioned fear assessed by freezing and by the suppression of three different baselines. Anim. Learn. Behav. 8:429-434.

Brener,J. and Goesling,W.J. 1970. Avoidance conditioning of activity and immobility in rats. J. Comp. Physiol. Psychol. 70:276-280.

Brown,J.S. and Jacobs,A. 1949. The role of fear in the motivation and acquisition of responses. J. Exp. Psychol. 39:747-759.

Brown,J.S., Kalish,H.I., and Farber,I.E. 1951. Conditioned fear as revealed by magnitude of startle response to an auditory stimulus. J. Exp. Psychol. 41:317-328.

Cahill,L., McGaugh,J.L., and Weinberger,N.M. 2001. The neurobiology of learning and memory: some reminders to remember. Trends. Neurosci. 24:578-581.

Cahill,L., Vazdarjanova,A., and Setlow,B. 2000. The basolateral complex is involved with, but is not necessary for, rapid acquisition of Pavlovian 'fear conditioning'. Eur. J. Neurosci. 12:3044-3050.

Cahill,L., Weinberger,N.M., Roozendaal,B., and McGaugh,J.L. 1999. Is the amygdala a locus of "conditioned fear"? Some questions and caveats. Neuron 23:327-328.

Campbell,B.A. and Campbell,E.H. 1962. Retention and extinction of learned fear in infant and adult rats. J. Comp. Physiol. Psychol. 55:1-8.

Cimadevilla,J.M., Fenton,A.A., and Bures,J. 2000. Functional inactivation of dorsal hippocampus impairs active place avoidance in rats. Neurosci. Lett. 285:53-56.

Coleman-Mesches,K. and McGaugh,J.L. 1995. Muscimol injected into the right or left amygdaloid complex differentially affects retention performance following aversively motivated training. Brain Res. 676:183-188.

Davis,M. 1997. Neurobiology of fear responses: the role of the amygdala. J. Neuropsychiatry Clin. Neurosci. 9:382-402.

Davis,M. 2000. The role of the amygdala in conditioned and unconditioned fear and anxiety. In The Amygdala: Second Edition: A Functional Analysis (ed. J. P. Aggleton), pp. 213-287. Oxford University Press, Oxford, UK.

Decker,M.W., Curzon,P., and Brioni,J.D. 1995. Influence of separate and combined septal and amygdala lesions on memory, acoustic startle, anxiety, and locomotor activity in rats. Neurobiol. Learn. Mem. 64:156-168.

Donovick,P.J. 1974. A metachromatic stain for neural tissue. Stain Tech. 49:49-51.

Edeline,J.-M., Hars,B., Hennevin,E., and Cotillon,N. 2002. Muscimol diffusion after intracerebral microinjections: a reevaluation based on electrophysiological and autoradiographic quantifications. Neurobiol. Learn. Mem. 78:100-124.

Everitt,B.J., Cardinal,R.N., Hall,J., Parkinson,J.A., and Robbins,T.W. 2000. Differential involvement of amygdala subsystems in appetitive conditioning and drug addiction. In The Amygdala: Second Edition: A Functional Analysis (ed. J. P. Aggleton), pp. 353-390. Oxford University Press, Oxford, UK.

Fanselow,M.S. 1980. Conditional and unconditional components of postshock freezing. Pav. J. Biol. Sci. 15:177-182.

Fanselow,M.S. 1984. What is conditioned fear? Trends. Neurosci. 7:460-462.

Fanselow,M.S. and Gale,G.D. 2003. The amygdala, fear, and memory. Ann. N. Y. Acad. Sci. 985:125-134.

Fanselow,M.S. and Kim,J.J. 1994. Acquisition of contextual Pavlovian fear conditioning is blocked by application of an NMDA receptor antagonist D,L-2-Amino-5-Phosphonovaleric Acid to the basolateral amygdala. Behav. Neurosci. 108:210-212.

Fanselow,M.S. and LeDoux,J.E. 1999. Why we think plasticity underlying Pavlovian fear conditioning occurs in the basolateral amygdala. Neuron 23:229-232.

Fendt,M. 2001. Injections of the NMDA receptor antagonist aminophosphonopentanoic acid into the lateral nucleus of the amygdala block the expression of fear-potentiated startle and freezing. J. Neurosci. 21:4111-4115.

Fendt,M. and Fanselow,M.S. 1999. The neuroanatomical and neurochemical basis of conditioned fear. Neurosci. Biobehav. Rev. 23:743-760.

Gaston,M.G. and Freed,L. 1969. Effect of amygdaloid lesions in a fear conditioning situation not involving instrumental learning. Psychon. Sci. 16:55-56.

Goldstein,M.L. 1960. Acquired drive strength as a joint function of shock intensity and number of acquisition trials. J. Exp. Psychol. 60:349-358.

Goldstein,M.L. 1968. Effects of lesions of the amygdaloid complex on peripheral shock thresholds and activity in the hooded rat. The Journal of General Psychology 79:59-74.

Goosens,K.A. and Maren,S. 2001. Contextual and auditory fear conditioning are mediated by the lateral, basal, and central amygdaloid nuclei in rats. Learn. Mem. 8:148-155.

Helmstetter,F.J. 1992. Contribution of the amygdala to learning and performance of conditional fear. Physiol. Behav. 51:1271-1276.

Helmstetter,F.J. and Bellgowan,P.S. 1994. Effects of muscimol applied to the basolateral amygdala on acquisition and expression of contextual fear conditioning in rats. Behav. Neurosci. 108:1005-1009.

Holahan,M.R. and White,N.M. 2002. Conditioned memory modulation, freezing, and avoidance as measures of amygdala-mediated conditioned fear. Neurobiol. Learn. Mem. 77:250-275.

Holahan,M.R. and White,N.M. 2003. Effect of muscimol inactivation of the basolateral or central amygdala on shock-conditioned responses. Ann. N. Y. Acad. Sci. 985:525-527.

Isaacson,R.L., Douglas,R.J., and Moore,R.Y. 1961. The effect of radical hippocampal ablation on acquisition of avoidance response. J. Comp. Physiol. Psychol. 54:625-628.

Iwata,J., Chida,K., and LeDoux,J.E. 1987. Cardiovascular responses elicited by stimulation of neurons in the central amygdaloid nucleus in awake but not anesthetized rats resemble conditioned emotional responses. Brain Res. 418:183-188.

Kapp,B.S., Gallagher,M., Underwood,M.D., McNall,C.L., and Whitehorn,D. 1982. Cardiovascular responses elicited by electrical stimulation of the amygdala central nucleus in the rabbit. Brain Res. 234:251-262.

Katoaka,Y., Shibata,K., Yamashita,K., and Ueki,S. 1987. Differential mechanisms involved in the anticonflict action of benzodiazepines injected into the central amygdala and mammillary body. Brain Res. 416:243-247.

Kesner,R.P. 1998. Neurobiological views of memory. In Neurobiology of Learning and Memory (ed. J. L. Martinez and R. P. Kesner), pp. 361-416. Academic Press, San Diego, CA.

Kesner,R.P. and Gilbert,P.E. 2001. Process-oriented view of amygdala and hippocampus. Mediation of reward value and spatial location information. In Memory Consolidation: Essays in Honor of James L. McGaugh (ed. P. E. Gold and W. T. Greenough), pp. 249-273. American Psychological Association, Washington, D.C..

Killcross,S., Robbins,T.W., and Everitt,B.J. 1997. Different types of fear-conditioned behaviour mediated by separate nuclei within the amygdala. Nature 388:377-380.

Kim,J.J. and Fanselow,M.S. 1992. Modality-specific retrograde amnesia of fear. Science 256:675-677.

Kim,J.J., Rison,R.A., and Fanselow,M.S. 1993. Effects of amygdala, hippocampus, and periacqueductal gray lesions on short- and long-term contextual fear. Behav. Neurosci. 107:1093-1098.

Kirkby,R.J. and Kimble,D.P. 1968. Avoidance and escape behavior following striatal lesions in the rat. Exp. Neurol. 20:215-227.

Kirkby,R.J. and Polgar,S. 1974. Active avoidance in the laboratory rat following lesions of the dorsal or ventral caudate nucleus. Physiol. Psychol. 2:301-306.

Kumar,R. 1970. Effects of fear on exploratory behaviour in rats. Q. J. Exp. Psychol. 22:205-214.

LeDoux,J. 1998. Fear and the brain: where have we been, and where are we going? Biol. Psychiatry 44:1229-1238.

LeDoux,J. 2000a. The amygdala and emotion: a view through fear. In The Amygdala: Second Edition: A Functional Analysis (ed. J. P. Aggleton), pp. 289-310. Oxford University Press, Oxford, UK.

LeDoux,J.E. (1996). Emotional networks and motor control: a fearful view. In Progress in Brain Research (ed. G. Holstege, R. Bandler, and C. B. Saper), pp. 437-446. Elsevier Science, New York, NY.

LeDoux,J.E. 2000b. Emotion circuits in the brain. Annu. Rev. Neurosci. 23:155-184.

Lee,H.J., Choi,J.-S., Brown,T.H., and Kim,J.J. 2001. Amygdalar NMDA receptors are critical for the expression of multiple conditioned fear responses. J. Neurosci. 21:4116-4124.

Lee,Y., Walker,D.L., and Davis,M. 1996. Lack of a temporal gradient of retrograde amnesia following NMDA-induced lesions of the basolateral amygdala assessed with the fear-potentiated startle paradigm. Behav. Neurosci. 110:836-839.

Maren,S. 1998. Overtraining does not mitigate contextual fear conditioning deficits produced by neurotoxic lesions of the basolateral amygdala. J. Neurosci. 18:3088-3097.

Maren,S. 1999. Neurotoxic basolateral amygdala lesions impair learning and memory but not the performance of conditional fear in rats. J. Neurosci. 19:8696-8703.

Maren,S. 2001. Neurobiology of Pavlovian fear conditioning. Annu. Rev. Neurosci. 24:897-931.

Maren,S. 2003. The amygdala, synaptic plasticity, and fear memory. Ann. N. Y. Acad. Sci. 985:106-113.

Maren,S., Aharonov,G., and Fanselow,M.S. 1996a. Retrograde abolition of conditional fear after excitotoxic lesions in the basolateral amygdala of rats: absence of a temporal gradient. Behav. Neurosci. 110:718-726.

Maren,S., Aharonov,G., and Fanselow,M.S. 1997. Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats. Behav. Brain Res. 88:261-274.

Maren,S., Aharonov,G., Stote,D.L., and Fanselow,M.S. 1996b. *N*-Methyl-D-Aspartate receptors in the basolateral amygdala are required for both acquisition and expression of conditional fear in rats. Behav. Neurosci. 110:1365-1374.

Martin,J.H. 1991. Autoradiographic estimation of the extent of reversible inactivation produced by microinjection of lidocaine and muscimol in the rat. Neuroscience 127:160-164.

Maxwell,S.E. and Delaney,H.D. 1990. Designing experiments and analyzing data., pp. 170-206. Brooks/Cole Publishing Co., Pacific Grove, CA.

McAllister,W.R. and McAllister,D.E. 1962. Role of CS and of apparatus cues in the measurement of acquired fear. Psychol. Rep. 11:749-756.

McAllister,W.R. and McAllister,D.E. 1971. Behavioral measurement of conditioned fear. In Aversive Conditioning and Learning (ed. F. R. Brush), pp. 105-179. Academic Press, San Diego, CA.

McAllister,W.R. and McAllister,D.E. 1995. Two-factor fear theory: implications for understanding anxiety-based clinical phenomena. In Theories of Behavior Therapy: Exploring Behavior Change (ed. W. O'Donohue and L. Krasner), pp. 145-171. American Psychological Association, Washington, D.C.

McGaugh,J.L., Cahill,L., and Roozendaal,B. 1996. Involvement of the amygdala in memory storage: interaction with other brain systems. Proc. Natl. Acad. Sci. U. S. A. 93:13508-13514.

McGaugh,J.L., Ferry,B., Vazdarjanova,A., and Roozendaal,B. 2000. Amygdala: role in modulation of memory storage. In The Amygdala: Second Edition: A Functional Analysis (ed. J. P. Aggleton), pp. 391-423. Oxford University Press, Oxford, UK.

McNish,K.A., Gewirtz,J.C., and Davis,M. 1997. Evidence of contextual fear after lesions of the hippocampus: a disruption of freezing but not fear-potentiated startle. J. Neurosci. 17:9353-9360.

Miller,N.E. 1948. Studies of fear as an acquirable drive: I. Fear as motivation and fear-reduction as reinforcement in the learning of new responses. J. Exp. Psychol. 38:89-101.

Mowrer,O.H. 1947. On the dual nature of learning -- A re-interpretation of "conditioning" and "problem-solving". Harv. Edu. Rev. 17:102-148.

Mowrer,O.H. and Lamoreaux,R.R. 1946. Fear as an intervening variable in avoidance conditioning. J. Comp. Psychol. 39:29-50.

Muller,J., Corodimas,K.P., Fridel,Z., and LeDoux,J.E. 1997. Functional inactivation of the lateral and basal nuclei of the amygdala by muscimol infusion prevents fear conditioning to an explicit conditioned stimulus and to contextual stimuli. Behav. Neurosci. 111:683-691.

Packard,M.G. and Cahill,L. 2001. Affective modulation of multiple memory systems. Curr. Opin. Neurobiol. 11:752-756.

Packard,M.G., Cahill,L., and McGaugh,J.L. 1994. Amygdala modulation of hippocampal-dependent and caudate nucleus-dependent memory processes. Proc. Natl. Acad. Sci. U. S. A. 91:8477-8481.

Packard,M.G. and Teather,L.A. 1998. Amygdala modulation of multiple memory systems: hippocampus and caudate-putamen. Neurobiol. Learn. Mem. 69:163-203.

Paxinos,G. and Watson,C. 1998. The rat brain atlas in stereotaxic coordinates. Academic Press, San Diego, CA.

Phillips,R.G. and LeDoux,J.E. 1992. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behav. Neurosci. 106:274-285.

Rescorla,R.A. and Solomon,R.L. 1967. Two-process learning theory: relationships between Pavlovian conditioning and instrumental learning. Psychol. Rev. 74:151-182.

Roberts,A.J., Cole,M., and Koob,G.F. 1996. Intra-amygdala muscimol decreases operant ethanol self-administration in dependent rats. Alcohol Clin. Exp. Res. 20:1289-1298.

Salinas,J.A. and McGaugh,J.L. 1996. The amygdala modulates memory for changes in reward magnitude: involvement of the amygdaloid GABAergic system. Behav. Brain Res. 80:87-98.

Salinas,J.A. and White,N.M. 2003. Differential contributions of the hippocampus, amygdala, and dorsal striatum to contextual and cued Pavlovian fear conditioning. (In press, Behav. Brain Res.).

Sanders,S.K. and Shekhar,A. 1991. Blockade of $GABA_A$ receptors in the region of the anterior basolateral amygdala of rats elicits increases in heart rate and blood pressure. Brain Res. 576:101-110.

Selden,N.R.W., Everitt,B.J., Jarrard,L.E., and Robbins,T.W. 1991. Complementary roles for the amygdala and hippocampus in aversive conditioning to explicit and contextual cues. Neuroscience 42:335-350.

Sidman,M. 1962. Classical avoidance without a warning stimulus. J. Exp. Anal. Behav. 5:97-104.

Suess,W.M. and Berlyne,D.E. 1978. Exploratory behavior as a function of hippocampal damage, stimulus complexity, and stimulus novelty in the hooded rat. Behav. Bio. 23:487-499.

Thielen,S.K. and Shekhar,A. 2002. Amygdala priming results in conditioned place avoidance. Pharmacol. Biochem. Behav. 71:401-406.

Vazdarjanova,A., Cahill,L., and McGaugh,J.L. 2001. Disrupting basolateral amygdala function impairs unconditioned freezing and avoidance in rats. Eur. J. Neurosci. 14:709-718.

Vazdarjanova,A. and McGaugh,J.L. 1998. Basolateral amygdala is not critical for cognitive memory of contextual fear conditioning. Proc. Natl. Acad. Sci. U. S. A. 95:15003-15007.

Viaud,M.D. and White,N.M. 1989. Dissociation of visual and olfactory conditioning in the neostriatum of rats. Behav. Brain Res. 32:31-42.

Wang,S. and Redgrave,P. 1997. Microinjections of muscimol into lateral superior colliculus disrupt orienting and oral movements in the formalin model of pain. Neuroscience 81:967-988.

White,N.M. and McDonald,R.J. 2002. Multiple parallel memory systems in the brain of the rat. Neurobiol. Learn. Mem. 77:125-184.

Wilensky,A.E., Schafe,G.E., and LeDoux,J.E. 1999. Functional inactivation of the amygdala before but not after auditory fear conditioning prevents memory formation. J. Neurosci. 19:1-5.

Wilensky,A.E., Schafe,G.E., and LeDoux,J.E. 2000. The amygdala modulates memory consolidation of fear-motivated inhibitory avoidance learning but not classical fear conditioning. J. Neurosci. 20:7059-7066.

Zangrossi,H. and File,S.E. 1992. Behavioral consequences in animals tests of anxiety and exploration of exposure to cat odor. Brain Res. Bull. 29:381-388.

Figure Legends

Figure 1. Representative sections with cannulae tracks. Sections were randomly taken from rats injected with saline or muscimol before context-shock pairings or before testing. Injector tips were typically located dorsal to the central amygdala.

Figure 2. Digital sections of the amygdala complex stained for c-Fos protein expression. Right and left hemispheres are shown from representative sections taken from a rat injected with muscimol or saline before the closed door test. c-Fos expression was elevated around the injector tip but declined rapidly in the rat injected with muscimol.

Figure 3. Quantitative analysis of the c-Fos data. Data are expressed as mean ± SEM. A. Left hemisphere. B. Right hemisphere. C-Fos expression was reduced in all regions examined (LA/BLA - lateral/basolateral amygdala; CeA - central amygdala; MeA - medial amygdala; Cortical - cortical amygdala and piriform cortex). The relatively small difference between the saline and muscimol injected rats in the CeA is most likely due to the elevation in c-Fos expression produced by the injector tip. * $p < 0.05$ main effect of group (saline vs muscimol).

Figure 4. Data collected during the 2 min pre-shock phase (PreSh), the 4 min shock phase (Sh) and during the 6 min closed door test (Test). A. Pretraining - rats were injected with saline (train-sal) or muscimol (train-mus) and shocked or saline (con-sal) and not shocked. ** $p < 0.01$ vs con-sal and train-mus during Sh phase. ++ $p < 0.01$ vs con-sal during Test. + $p < 0.05$ vs train-sal and con-sal during Test. B. Pretesting - rats were shocked and injected with saline (test-sal) or muscimol (test-mus) or not shocked and injected with saline (con-sal) before testing. ** $p < 0.01$ vs test-sal and test-mus during Sh phase. ++ $p < 0.01$ vs test-sal during Test

Figure 5. Open door test. Avoidance ratios were calculated as the time spent in the unpaired compartment minus the time spent in the paired compartment divided by the total time. Abbreviations as in Fig 4. A. Pretraining. ** $p < 0.01$ vs train-sal. B. Pretesting. ** $p < 0.01$ vs test-sal.

Figure 6. Open door test. Crossover rates are the number of times a rat moved between the two compartments minus 1 divided by the total session time minus the time of the first bout

in the paired compartment. Abbreviations as in Fig 4. A. Pretraining. ** $p < 0.01$ vs train-sal. B. Pretesting. ** $p < 0.01$ vs test-sal, ## $p < 0.01$ vs con-sal.

group	time of first paired bout (s)	total time freezing (s)	rats freezing/ total rats
pretraining			
train-sal	161.1 ± 93.4	59.1 ± 37.1	4/7
con-sal	19.0 ± 8.9	0 ± 0	0/7
train-mus	29.5 ± 10.1	0 ± 0	0/7
pretesting			
test-sal	31.4 ± 17.0	10.7 ± 10.7	1/7
con-sal	3.7 ± 0.8	0 ± 0	0/6
test-mus	11.4 ± 2.1	0 ± 0	0/7

Table 1. Data from the first bout in the paired compartment during the open door test. The first bout was ended by the first crossover from the paired to the unpaired compartment. Time of first bout (mean ± SEM) was recorded by the infrared detectors. An experimenter recorded the times at which the rats started and stopped freezing (mean ± SEM) using standard definitions. Rats freezing were defined as rats that froze for more than 10% of the total time of the first bout in the paired compartment (range 10% - 60%).

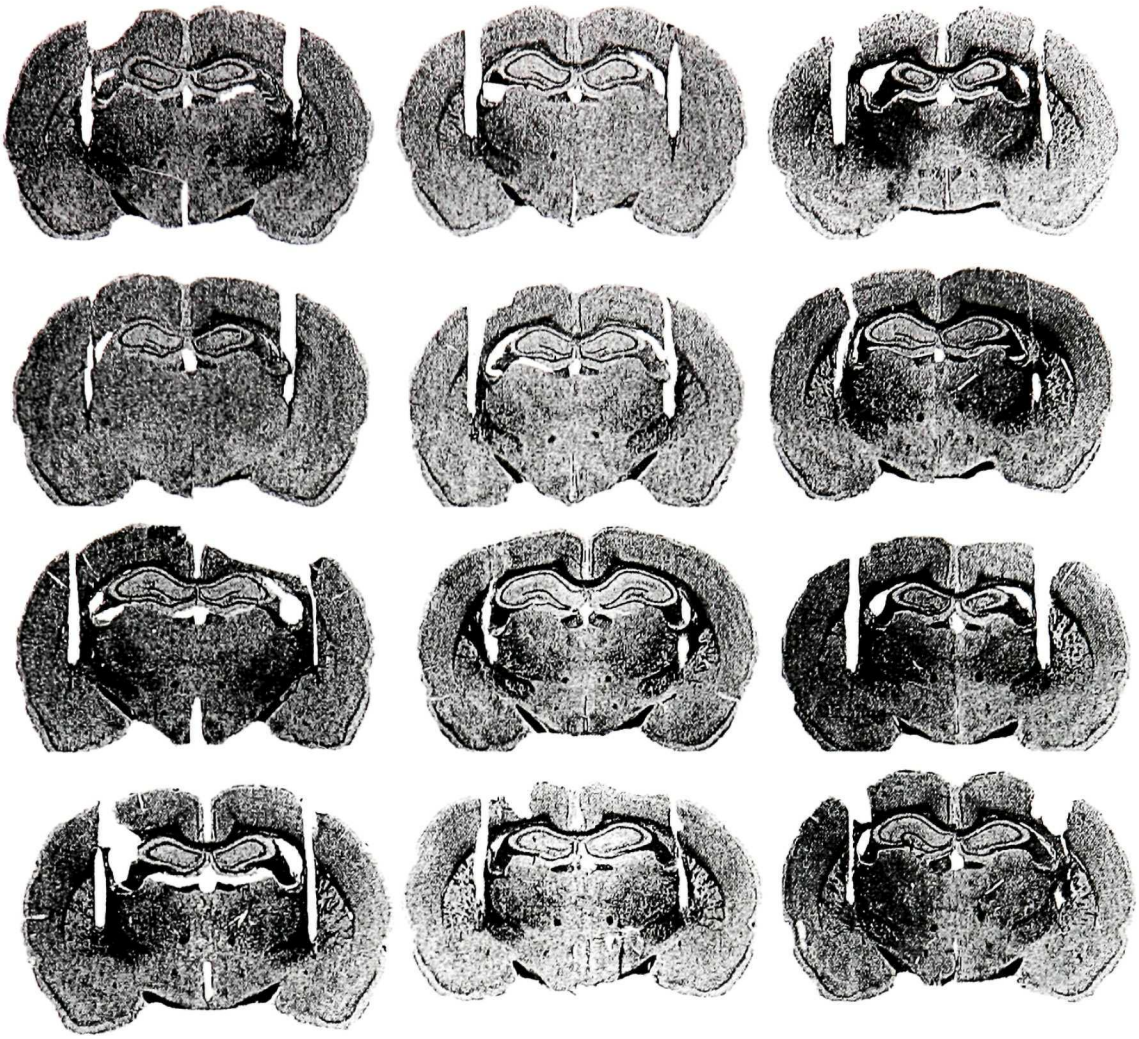
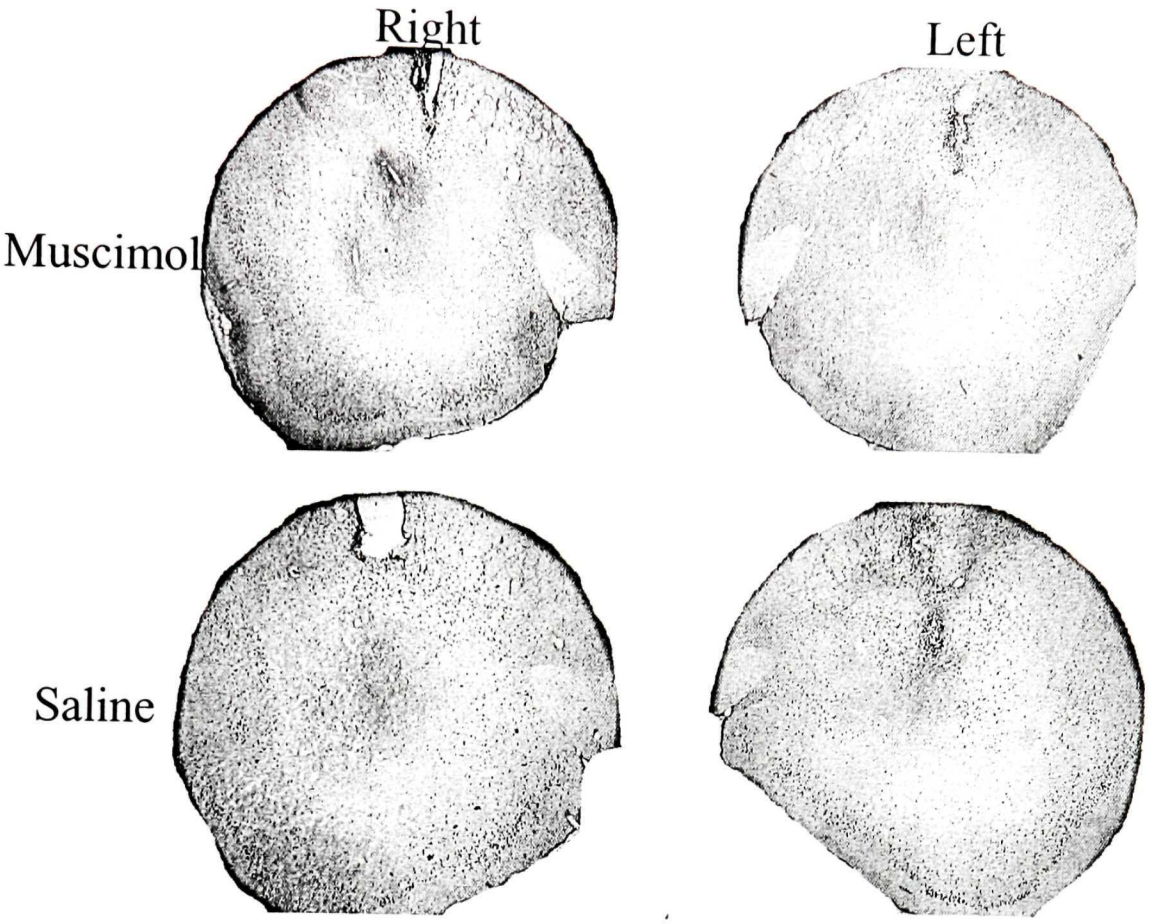


Figure 1 Holahan and White



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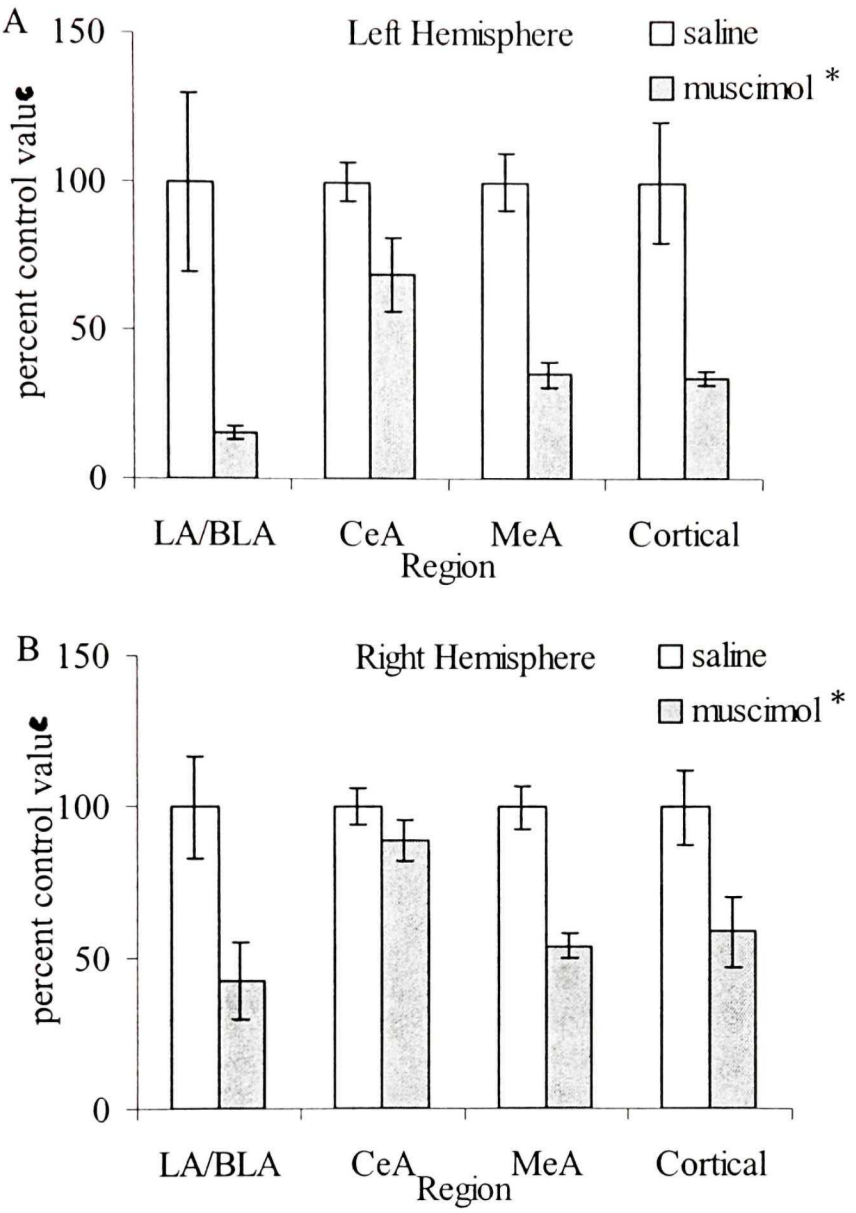

Figure 3 Holahan and White

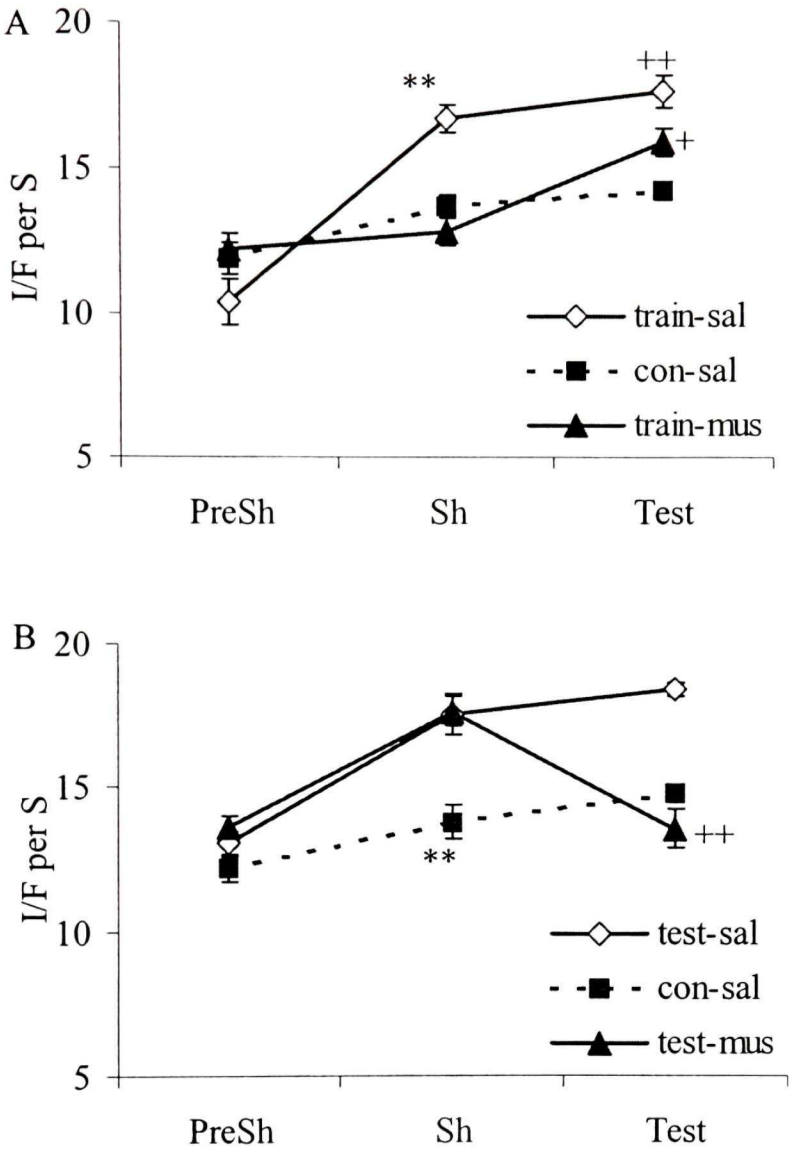

Figure 4 Holahan and White

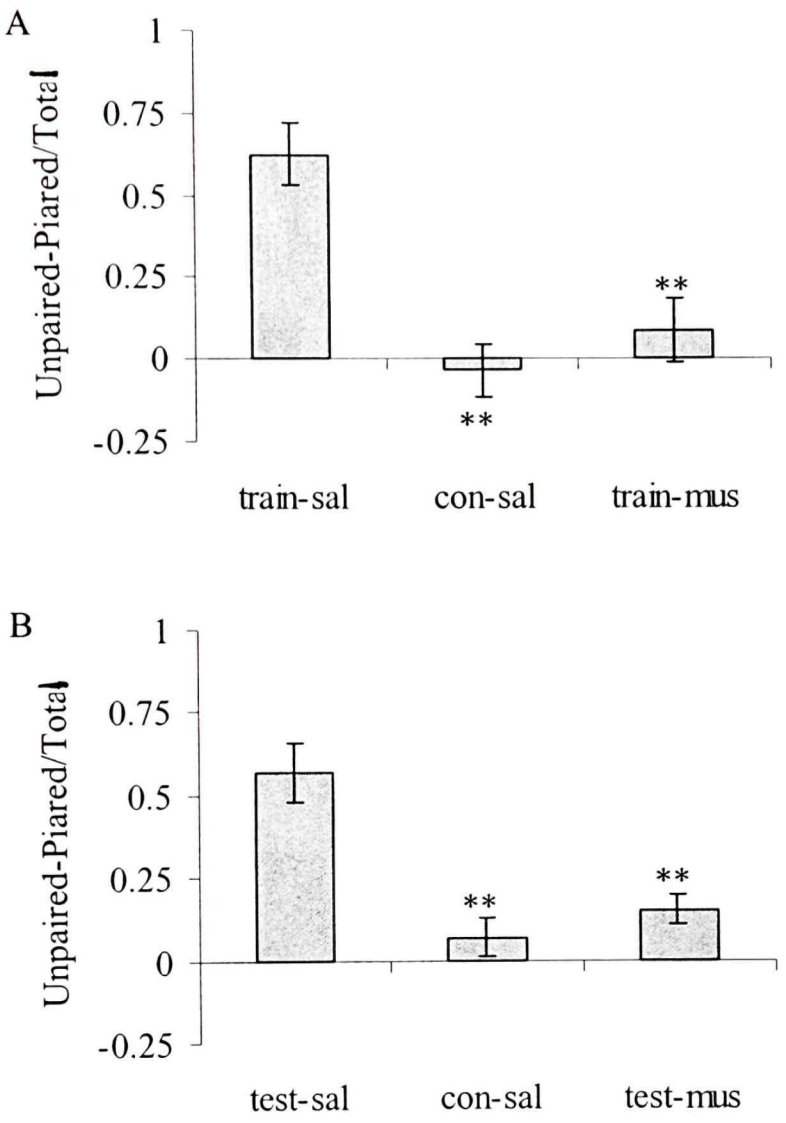

Figure 5 Holahan and White

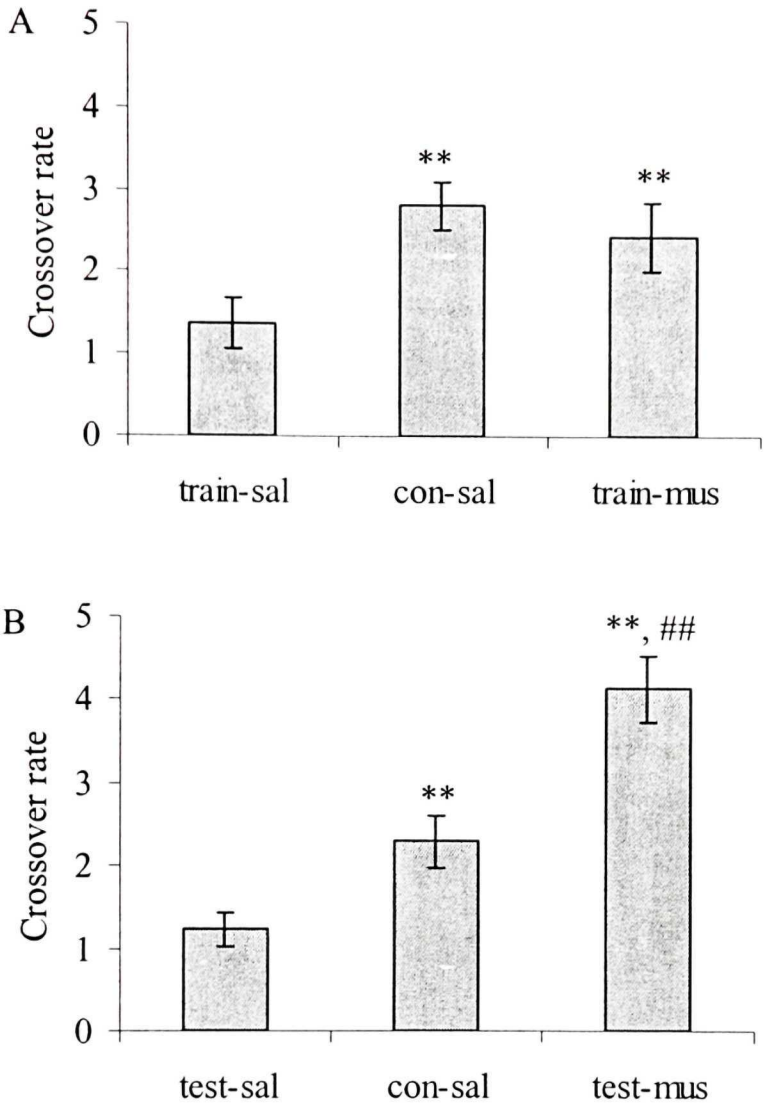

Figure 6 Holahan and White

Manuscript 3

Since freezing requires cessation of movement and avoidance requires its initiation, the effects of muscimol found in Manuscript 2 cannot be attributed to a deficit in behavior production. Inactivation of the amygdala may have affected production of a conditioned aversive (affective) state that promotes either freezing or place avoidance, depending on environmental constraints, during exposure to the CS. The postulated internal aversive state produced by exposure to unconditioned (US) and conditioned (CS) stimuli may consist of a set of internal responses involving some combination of neural activity, neurotransmitter, and hormone release. These responses require specialized procedures to be measured and are not normally observed during exposure to an aversive US or CS. However, a subset of these internal responses is known to modulate memory. Therefore, Manuscript 3 reports on rats that were given intra-amygdala saline or muscimol injections before exposure to conditioned aversive stimuli as a posttraining memory modulator. Blockade of conditioned memory modulation with intra-amygdala muscimol injections was hypothesized to reflect the elimination of an array of conditioned internal responses comprising an aversive affective state.

Amygdala Inactivation Blocks Expression of Conditioned Memory Modulation and the Promotion of Place Avoidance and Freezing

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Running head: Amygdala mediated responses

Abstract

Unconditioned (McGaugh, 2000) and conditioned (Holahan and White, 2002a) stimuli elicit amygdala-mediated, neural and/or hormonal responses that modulate memory. To examine how these internal responses are related to the behavioral effects of exposure to these stimuli, rats were given intra-amygdala saline or muscimol injections before exposure to conditioned aversive stimuli as a posttraining memory modulator for conditioned cue preference (CCP) training. Conditioned, saline-injected rats showed elevated freezing and place avoidance and enhanced CCP retention compared to unconditioned controls; these effects were absent in conditioned muscimol-injected rats. Amygdala inactivation blocked two incompatible behaviors (freezing and place avoidance) and the internal conditioned responses that modulated memory. This makes it unlikely that the amygdala itself generates the observed behaviors. When rats are exposed to an aversive CS the amygdala appears to produce an array of internal neural and hormonal responses (internal responses) that modulate memory and promote behaviors such as freezing and place avoidance, as determined by the environment. The occurrence of these effects in the presence of conditioned stimuli may reflect the existence of amygdala-mediated aversive Pavlovian associations.

Key words: amygdala, memory modulation, consolidation, avoidance, freezing, conditioned fear

Introduction

In aversive conditioning procedures, pairing a neutral stimulus (CS) with shock (US) is thought to produce aversive Pavlovian-type learning. Subsequently, the neural substrate representing this aversive association is thought to be activated during exposure to the CS, resulting in an unobservable conditioned aversive state. The existence of such a conditioned affective state and the Pavlovian learning that produces it is usually inferred from a change in the occurrence of overt behaviors such as freezing or avoidance (Davis, 1997; LeDoux, 1998; LeDoux, 2000; McAllister & McAllister, 1971).

Freezing has been described as a stereotypical, species-specific reaction to threatening stimuli (Bolles, 1970). The behavior has been operationalized as a cessation of motor activity including whisker and nose movements (Bolles & Collier, 1976), sitting rigidly motionless (Bindra & Anchel, 1963) except for movement necessitated by respiration (Fanselow, 1980). Freezing is increased in the presence of cues previously paired with shock and has been used to assess and study aversive associative processes (Bindra & Anchel, 1963; Blanchard & Blanchard, 1969; Bolles & Collier, 1976; Fanselow, 1980; LeDoux, 1996; McAllister & McAllister, 1971).

Avoidance occurs when a rat presses a lever or minimizes the time spent in a compartment to avoid exposure to an aversive CS (Olton, 1973; Wadenberg & Hicks, 1999). In contrast to freezing, avoidance is an active behavior. When an aversive environment includes an exit, rats move away from the aversive CS and increase the proportion of time they spend in accessible neutral locations (Antoniadis & McDonald, 1999; Blanchard & Blanchard, 1968; 1970a; 1970b; 1971; Campbell & Campbell, 1962; Holahan & White, 2002a; Kumar, 1970; McAllister & McAllister, 1962; Miller, 1948). This behavior often occurs for the first time in the presence of the aversive CS, meaning that it could not have been learned during conditioning. The appearance of such new behaviors leads to the inference of a conditioned aversive state and its underlying aversive Pavlovian association (Miller, 1948; Mowrer, 1947; Mowrer & Lamoreaux, 1946).

Both freezing and avoidance are elicited by shock-conditioned cues and both behaviors have been used to infer the existence of aversive internal states (Brown & Jacobs, 1949; McAllister & McAllister, 1971; Miller, 1948). However, the occurrence of these observable

behaviors is partly determined by the environment in which they are measured. If there is an escape route, freezing decreases and avoidance increases (Amorapanth et al., 2000; Blanchard & Blanchard, 1971). Freezing has also been found to interfere with the expression of avoidance (Anisman, 1973; Anisman & Waller, 1972; 1973). Both of these findings may confound the relationship between the behavior observed and the inferred aversive state (cf. McAllister & McAllister, 1971; Rescorla & Solomon, 1967).

The postulated internal aversive state produced by exposure to unconditioned (US) and conditioned (CS) stimuli may consist of a set of internal responses involving some combination of neural activity, neurotransmitter, and hormone release. These responses require specialized procedures to be measured and are not normally observed during exposure to an aversive US or CS. However, a subset of these internal responses is known to modulate memory (Cahill, 2000; Cahill & McGaugh, 1996; Gold & McGaugh, 1975; Gold & van Buskirk, 1975; McGaugh, 2000; McGaugh & Cahill, 1997; White, 1998). Examining the memory modulating effects of a CS can provide a different measure for studying a putative internal state independently of immediately occurring behaviors that may be promoted by the state. Modulating effects of conditioned stimuli are observed at a later time in a different apparatus as effects on the retention of previously acquired information (Cahill & McGaugh, 1996; McGaugh, 2000).

Posttraining exposure to an aversive US has been shown to modulate memory expressed as enhanced suppression of drinking during presentation of a tone CS (White & Legree, 1984), step-through inhibitory avoidance (Jodar et al., 1996), and an appetitive discrimination on a Y-maze (Holahan & White, 2002a). Holahan and White (2002a) also found that posttraining exposure to an aversive CS modulated memory for the same discrimination. In these experiments, posttraining exposure to unconditioned foot shock (US) or to conditioned stimuli (CS) associated with the US is inferred to have produced a set of unconditioned (UR) or conditioned (CR) internal responses which modulated memory. As with freezing and avoidance produced by exposure to a CS, conditioned memory modulation suggests the existence of an underlying aversive Pavlovian association.

It has been suggested (Davis, 2000; Fendt & Fanselow, 1999; LeDoux, 2000) that the amygdala mediates a conditioned affective state following aversive conditioning. This

hypothesis is partly based on inferences made from observations of freezing in the presence of shock-paired CSs. Elevated freezing during exposure to a compartment previously paired with shock is attenuated by pre-training electrolytic lesions of the amygdala complex (Phillips & LeDoux, 1992), radio-frequency lesions of the medial (MeA) amygdala (Blanchard & Blanchard, 1972), electrolytic lesions of the central (CeA) or basolateral (BLA) nuclei (Holahan & White, 2002a; Kim et al., 1993), and by NMDA lesions centered on the BLA (Cahill et al., 2000; Lee et al., 1996; Maren et al., 1996; Maren, 1998;1999; Vazdarjanova et al., 2001; Vazdarjanova & McGaugh, 1998) and the CeA or LA (Goosens & Maren, 2001).

Temporary inactivation of the amygdala has also been shown to impair acquisition and expression of freezing behavior. Pretraining (Fanselow & Kim, 1994) or pretesting (Fendt, 2001; Lee et al., 2001; Maren et al., 1996) injection of the NMDA antagonist APV reduces freezing during re-exposure to an aversive CS. The $GABA_A$ agonist muscimol also attenuates freezing when injected into the BLA before training or testing (Helmstetter & Bellgowan, 1994; Muller et al., 1997; Wilensky et al., 1999).

One argument concerning freezing deficits following amygdala lesions is that the rats might be unable to perform the specific behavior (Cahill et al., 1999; Cahill et al., 2000; Cahill et al., 2001; Vazdarjanova & McGaugh, 1998) yet retain the underlying aversive association. Avoidance, a behavior that is incompatible with freezing, provides an alternative behavioral index of an aversive internal state. Both electrolytic (Gaston & Freed, 1969) and NMDA (Antoniadis & McDonald, 2000) lesions of the amygdala complex block avoidance of a shock-conditioned context. Pretraining or pretesting intra-amygdala injections of muscimol (Holahan & White, 2001) also block avoidance. Lesions of BLA but not CeA impaired rats' ability to press a lever to avoid presentation of an aversive CS (Killcross et al., 1997) and escape from a shock-conditioned tone (Amorapanth et al., 2000). However, several studies have failed to show that BLA lesions block active forms of avoidance (Ambrogi Lorenzini et al., 1991; Holahan & White, 2002a; Selden et al., 1991; Vazdarjanova & McGaugh, 1998) while others (Holahan & White, 2002a; 2002b; Poremba & Gabriel, 1997; Smith et al., 2001) report that damage to the CeA results in avoidance deficits. These findings indicate that avoidance is impaired by combined lesions of the BLA and CeA, while lesions more-or-less

confined to one of these areas produce less consistent results.

The amygdala also mediates the posttraining memory modulation effect produced by unconditioned and conditioned stimuli. Electrolytic lesions of the BLA/CeA block the modulating action of posttraining bicuculline and the memory-impairing effect of posttraining muscimol on the retention of an inhibitory avoidance task (Ammassari-Teule et al., 1991). NMDA lesions of the amygdala complex (Cahill & McGaugh, 1991) block the enhancing effect of posttraining epinephrine on retention of inhibitory avoidance (however, these lesions also blocked unmodulated retention). Ibotenic acid lesions of MeA or BLA, but not CeA, blocked the modulating effect of systemic posttraining injections of dexamethosone on inhibitory avoidance (Roozendaal & McGaugh, 1996). The modulating action of dexamethosone is also blocked by BLA injections of adrenergic antagonists (Quirarte et al., 1997) or atropine (Power et al., 2000). The modulating action of oxotremorine is also blocked by CeA injections of atropine (Introini-Collison et al., 1996). Electrolytic lesions of CeA or MeA but not BLA or LA block the memory modulating effect of posttraining exposure to an aversive CS on an appetitive Y-maze discrimination (Holahan & White, 2002a). These findings suggest that several amygdala nuclei may mediate the memory modulating action of both unconditioned and conditioned posttraining treatments.

In the present study three behaviors (freezing, avoidance, and memory modulation) produced by exposure to a set of shock-conditioned contextual cues were measured in the same rats. This allowed a comparison of behaviors used to infer the existence of an internal aversive state. In Experiment 2 the effect of intra-amygdala muscimol injections on the expression of these behaviors during exposure to the shock-conditioned contextual cues was investigated.

EXPERIMENT 1

In a previous study (Holahan & White, 2002a), posttraining exposure to a shock-conditioned context or tone modulated the retention of an appetitive Y-maze discrimination task that required four days of training. The present study was designed to develop a to-be modulated task that could be acquired in a single training session. A variant of the radial maze conditioned cue preference (CCP) task (McDonald & White, 1995a; 1995b) was used in which rats were moved by the experimenter between food-paired and unpaired arms (White

& Ouellet, 1997). Posttraining exposure to the shock-conditioned cues consisted of placing rats in the shuttle-box with the door to the neutral compartment open, allowing them to move freely between the paired and unpaired compartments. In Holahan and White (2002a), rats were confined to the shock-paired context during posttraining exposure, providing a measure of freezing. The present design provided simultaneous measures of freezing, avoidance & locomotor activity during exposure to the CS. Conditioned modulation produced by this posttraining exposure was assessed 24 hours later with a CCP test on the maze.

Materials and Methods

Subjects

Subjects were 52 male Long-Evans rats (Charles River, St. Constant, Québec, Canada) that weighed 250 - 275 g at the start of the experiment. They were housed in individual cages with free access to water. The temperature (22° C) and lighting (lights on: 0700 to 1900) of the animal housing unit were controlled. Care of the animals conformed with guidelines set by the Canadian Council on Animal Care.

Apparatus

The apparatus was located in a windowless 2.8 x 3.7 x 2.8 m (w x l x h) room. The room was partitioned with a sound attenuating divider (1.2 m long) that created a 2.8 x 2.3 m area for an 8-arm radial maze (described below). The room contained a number of distal cues including a rust colored carpeted wall with a yellow road sign and a white lab coat, a poster, several geometric shapes on the room divider, a black sheet draped over three shelves, a red door with a white lab coat hanging on it, and a video camera above the maze. There was also a brown table along one wall below the black sheet.

The conditioned preference apparatus was a wooden eight-arm radial maze painted flat gray consisting of an octagonal central platform 29.3 cm edge to edge with arms 42.8 cm long and 9 cm wide. Each arm was surrounded by a wall 15.8 cm high at the entrance, decreasing to 5 cm at the distal end. The surface of the maze was 54 cm from the floor. Wooden blocks (30 cm high) also painted flat gray were placed in the entrances of unused arms. Similar blocks with wooden panels attached (28 cm wide) were used to confine a rat to a 35 cm^2 area at the end of an arm during training. The panels confined a rat's view of the room to an arc of approximately 180° facing away from the maze.

The shuttle-box and controlling equipment were located behind the partition in the same room as the maze but were not visible from the maze. The shuttle-box consisted of two adjacent stainless steel compartments (29 x 28 x 24 cm) with clear Plexiglas front doors and a doorway in the center of the common wall that could be opened or closed using a guillotine-type opaque door. The walls of one compartment were grey; the walls of the other were black and white checkered. The floors of both compartments consisted of stainless steel rods connected to shock generators. Each rod was 0.5 cm in diameter with a distance between each rod of 1.5 cm center to center. The shuttle-box rested on two stainless steel catch pans 6 cm below the rod floor. It was inside a particle board shell (0.5 x 0.95 x 0.65 m) that had no front doors and rested on a 1.0 m high cabinet located in an alcove (1.15 x 0.95 x 2.0 m) that contained sound dampening materials

For each rat one compartment served as the paired side (in which shock was given) and the other as the unpaired side (in which no shock was given). When a compartment was unpaired the rod floor was covered with a 0.5 cm wire mesh. Rats do not show any consistent unconditioned side preferences in this apparatus (Holahan & White, 2002a).

A passive infra-red motion detector (Radio Shack, Model No. 49-550) modified to be optimally sensitive to the infrared wavelength emitted by rodents (R.E. Brown, personal communication) was mounted over a 6 cm diameter hole in the top of each compartment. The detectors were used to determine the amount of time a rat spent in each compartment and the number of times the rats moved between compartments.

A third, freestanding box (Box C) that measured 29 cm x 28 cm x 24 cm was also used. It was located on the brown table in the room and was visible from the maze. The frame of Box C was made of wood, the walls and ceiling were made from 1.0 cm wire mesh, and the floor was a flat metal sheet.

Procedure

Rats were handled by the experimenter on each of 5 days in the animal housing room. Groups of 7 - 8 rats were placed into a plastic handling box (70 x 54 x 33 cm) with wood chips covering the floor for 2 h per day while the experimenter picked up and held each rat for 5 min. During these 5 days, food was removed from the rats' cages. When returned to its home cage after handling, each rat was given 10 Froot Loops (Kellogg's, Battle Creek,

MI) and approximately 5 g of rat chow. At the end of the handling period, all rats weighed 85% ± 3% of their initial free feeding weights. They were maintained at this level of deprivation with rat chow only throughout the remainder of the experimental procedure.

Aversive conditioning

On the two days following the handling period, 29 rats were individually exposed to Box C for 15 min per day. The next day, these rats were pre-exposed to the shuttle-box compartments with the door between the two compartments closed. For each rat, one compartment was randomly assigned as its "paired" compartment and the other became its "unpaired" compartment. These assignments were counterbalanced within groups. Each rat was placed into its paired compartment (rod floor exposed) for 6 min and then immediately placed into its unpaired compartment (wire mesh covering floor) for 6 min. No shocks were given in either compartment.

The following day, each of 22 rats was placed into its paired compartment and after 2 min, received 4, 0.5 sec, 1.0 mA shocks with a 1 min inter-shock interval. The 7 remaining rats were placed into their paired compartments and remained there for 6 min with no shock. The next day, each of the 29 rats was placed into its unpaired compartment for 6 min with no shock.

While the rats were in their paired compartments, an experimenter recorded the times at which the rats started and stopped freezing. Freezing was defined as the absence of all body movements except that produced by respiration (Bolles & Collier, 1976; Fanselow, 1980). The total time (in seconds) spent freezing was divided by 360 (6 min) and multiplied by 100 to obtain a percent freezing score.

Conditioned Cue Preference (CCP) training

CCP training on the radial maze began 24 h after exposure to the unpaired compartment for the 29 rats that received shock-training or 24 h after the fifth handling day for the 23 rats that were not trained. Each rat was randomly assigned to food-paired and food-unpaired arms separated by at least 2 other arms on the radial maze. Each training trial consisted of confining a rat on its unpaired arm for 5 min and then moving it to its paired arm for 5 min (White & Ouellet, 1997). When a rat was moved between arms, the experimenter opened the testing room door, entered the room, lifted the rat off the arm, placed it onto the other arm,

exited the room, and closed the door. Paired arms contained 30 Froot Loops, unpaired arms were empty. The number of Froot Loops remaining in the paired arm was counted at the end of CCP training and subtracted from 30 to provide a measure of consumption.

Training trials were given continuously within a single session. The 23 rats that were not given aversive conditioning received 1 (n = 8; group 1TT), 2 (n = 7; group 2TT), or 3 (n = 8; group 3TT) training trials with no posttraining treatments. The 29 rats that were given aversive conditioning all received 2 training trials. Each rat was returned to its home cage when its CCP training trials were complete.

Posttraining treatments

The 29 rats that underwent aversive conditioning were removed from their home cages at different times after CCP training and were placed into the shock-paired compartment with the connecting doorway to the unpaired compartment open. Each rat was allowed to move freely between the two compartments for 12 min. The time spent by each rat in each compartment and the number of times each rat moved between the compartments were recorded automatically by the infrared detectors.

Freezing was measured (as already described) during each rat's first bout in the paired compartment (ie, from the time the rat was first placed in its shock-paired compartment until it exited to the unpaired compartment for the first time). The total time each rat spent freezing during this first bout was divided by the duration of the bout and multiplied by 100 to obtain a percent freezing score.

A group of rats that had been shocked-trained (n = 8; group 15Sh) and the group that had not been shocked during training (n = 7; group 15NSh) were exposed to the shuttle-box 15 min after preference training. Additional groups of shock-trained rats were exposed to the shuttle-box 30 min (n = 7; group 30Sh) or 2 hours (n = 7; group 2HrSh) after preference training.

Conditioned preference test

Twenty four hours after preference training or the posttraining treatments, each rat was placed on the center platform of the maze for 20 min with the food-paired and food-unpaired arms open. There was no food in either arm. The entrances to the other arms were blocked. The times of entry into and exit from each arm were recorded and used to calculate the total

time spent in each arm. An entry into or an exit from an arm was scored when a rat's shoulders crossed an imaginary plane separating the arm from the central platform (McDonald & White, 1995a; 1995b).

Statistical analyses

Percent freezing scores were analyzed with a two-way repeated measures (group by day) ANOVA. Fisher's post hoc least significant difference tests (LSD) were applied to examine the locus of significant interactions (Maxwell & Delaney, 1990).

Avoidance ratios were calculated as the total amount of time spent in the unpaired compartment minus the amount of time in the paired compartment divided by the sum of these two times [(unpaired - paired)/ (unpaired + paired)]. Since a significant portion of the first bout in the paired compartment for many rats consisted of freezing and/or other forms of inactivity, the data for this bout were removed from the total time in the paired compartment used in this calculation. As freezing was both rare and brief when it did occur after the first bout, this provided a measure of avoidance that was almost completely free of interference from freezing. The mean durations of the first paired bout that were eliminated from the 12 min test were 25.2 ± 6.1 s (mean ± SEM) for group 15NSh, 80.4 ± 51.2 for group 15Sh, 133.9 ± 80.0 for group 30Sh, and 176.9 ± 76.2 for group 2HrSh. The avoidance ratios were analyzed with a randomized one way ANOVA and Fisher's LSD post hoc tests.

Movement between the two compartments during the conditioned posttraining treatments was expressed as a crossover rate, defined as the total number of times a rat moved between the paired and unpaired compartments divided by the session time remaining after subtraction of the duration of the first bout in the paired compartment. In these calculations, the number of crossovers was reduced by 1 to correct for elimination of the first bout, which was ended by the first crossover. Crossover rates were analyzed with a randomized one way ANOVA and Fisher's LSD post hoc tests.

For the conditioned preference data, the statistical comparison of interest was the difference between the mean times spent in the paired and unpaired arms within each experimental group. Accordingly, these times were compared using pairwise planned comparisons (Kirk, 1969, p. 73) following a two-way ANOVA (group by arm) with one repeated measure. The F value of each planned comparison was the difference between the

mean time in each arm squared, multiplied by the n per group, and divided by the mean square error of the interaction term from the ANOVA.

Scatterplots were generated to compare the behavioral measures obtained during the posttraining treatment with the conditioned preference test data. Percent freezing during the first paired bout, avoidance ratios, and crossover rates were compared to the difference in the amounts of time spent in the paired and unpaired arms during CCP testing. Data included in this analysis were from groups 15Sh and 30Sh, the groups that exhibited the modulation effect.

Results

The data from the groups that were given different numbers of training trials during CCP training are shown in Figure 1A. There were no CCPs in the groups given 1 or 2 training trials ($F(1,20) < 1.0$ for both groups); however there was a significant CCP in the group that received 3 training trials ($F(1,20) = 8.46$, $p < 0.01$). These findings led to the choice of 2 training trials for the modulation groups.

The CCP data for the groups that received posttraining exposure to the shuttle-box following 2 training trials are shown in Figure 1B. There was no CCP in group 15NSh ($F(1,25) < 1.0$), but there were significant CCPs in groups 15Sh ($F(1,25) = 53.29$, $p < 0.001$) and 30Sh ($F(1,25) = 14.13$, $p < 0.001$). There was no CCP in group 2HrSh ($F(1,25) < 1.0$). These results suggest that posttraining exposure to the conditioned cue had a retroactive modulating effect on memory rather than a proactive effect on performance.

The mean number of Froot Loops consumed during preference training for each of the posttraining treatment groups was analyzed with a randomized one-way ANOVA. There was no main effect of group ($F(3,25) < 1.0$).

Table 1 shows the percent freezing observed on the shock-training day and during the first bout in the paired compartment during the posttraining treatment. A significant main effect of group ($F(3,25) = 19.07$, $p < 0.001$) followed by post-hoc analyses showed that each shocked group (15Sh, 30SH, and 2HrSh) froze more than the no shock group ($p < 0.05$ for all comparisons) during both training and the first paired bout of the test. A significant main effect of day ($F(1,3) = 32.08$, $p < 0.001$) indicated that there was more freezing during shock training than during posttraining exposure to the shuttle-box in all groups ($p < 0.01$ for all

comparisons).

The avoidance ratios (Figure 2A) increased for all shocked groups, indicating that the rats in those groups spent more time in the unpaired than in the paired compartment. There was a significant main effect of group ($F(3,25) = 13.78$, $p < 0.0001$), and all shocked groups (15Sh, 30Sh, and 2HrSh) differed significantly from the no shock group (15NSh; t values > 3.5, $p < 0.01$ for all comparisons).

Figure 2B shows that the crossover rates for the shocked groups were lower than that for the no shock group during the test session ($F(3,25) = 10.14$, $p < 0.0001$). All shocked groups differed significantly from group 15NSh (t values > 3.0, $p < 0.01$ for all comparisons).

Figure 3 shows the scatterplots. Regression analysis confirmed the visual impression that there were no significant correlations between the CCP difference scores and percent freezing during the first paired bout ($r = 0.48$, $F(1,13) = 3.96$, $p = 0.07$), the conditioned avoidance ratios ($r = 0.14$, $F(1,13) < 1.0$), or the crossover rates ($r = 0.25$, $F(1,13) < 1.0$).

Discussion of Experiment 1

Exposure to shock-conditioned cues either 15 or 30 min posttraining enhanced retention of information required for expression of the CCP. Increasing the delay between maze training and the conditioned posttraining treatment to 2 hours eliminated this effect showing the classic time-dependent memory modulation effect (McGaugh, 1966). This replicates a previous finding (Holahan & White, 2002a).

The enhanced preference in the modulated groups was not due to a contingent association between the rats' experience on the maze and exposure to the shock-conditioned cues because the last arm the rat was exposed to on the maze was always the food-paired arm. Such an association would have resulted in less time spent in the food-paired arm during conditioned testing. The fact that the rats spent more time on the food-paired arm, together with the lack of enhanced modulation in the 2 hour delay group shows that the enhanced conditioned preference for the food-paired arm was due to a retroactive modulation of memory. The most important factor for producing the modulation effect, besides the delay between training and posttraining treatment, was the previous pairing between shock and the neutral cues, suggesting that it was produced by an aversive Pavlovian association.

In a previous experiment (Holahan & White, 1999), rats were confined to a single

compartment during shock training and during the conditioned test. In the present study, the door between the paired and unpaired compartments was closed during training but was open during the conditioned posttraining test. Freezing in these two conditions is compared in Table 1. Percent freezing was similar during closed door shock-training in both experiments, but was much higher during the closed door test than during the open door test. The change in the configuration of the apparatus influenced the amount of freezing observed. This could be due to the stimulus change produced by the open door (Claus & Bindra, 1960; Save et al., 1992; Suess & Berlyne, 1978) or to the availability of an exit from the paired compartment (Blanchard & Blanchard, 1971).

The fact that freezing occupied an average of less than 20% of the first bout in the paired compartment during the present open door test raises the question of what the rats did during the remaining 80% of the bout. Only one shocked rat (in the 2 hour delay group) escaped immediately after a period of freezing, giving a score of close to 100%. Eight rats did not freeze at all (see Fig 3A) and escaped almost immediately. The remaining rats initially froze and then began to display whisker and nose movements while still remaining motionless. Because these movements were observed, freezing was not recorded. Next, gross head movements typically occurred, followed by stretching toward the open door and finally, escape. Although these nose, whisker, and head movements mean that the behavior cannot be labeled freezing, they contrast sharply with the behavior of the no shock rats (and more than one-half of the shocked rats) and do not suggest normal, fear-free activity. Measures of immobility or decreases in activity that are not usually classified as freezing may also be indicative of an aversive affective state (Baron, 1964; Blanchard & Blanchard, 1969; Brener & Goesling, 1970; Kumar, 1970) in the rats that exhibit these behaviors.

New apparatus configurations are known to elicit exploration (Barnett, 1963) and aversive cues tend to decrease exploration (Baron, 1964; Blanchard & Blanchard, 1969; Brener & Goesling, 1970; Kumar, 1970). In the present experiment, exposure to the open door configuration for the first time during the posttraining treatment may have increased activity levels but these levels would have been decreased by the presence of an aversive internal state due to an aversive Pavlovian association with the paired compartment. This is consistent with the observation of decreased crossover rates in the shock-trained rats.

During posttraining exposure to the apparatus both shocked and non-shocked rats moved between the two compartments but shocked rats spent less time in the paired compartment than the non-shocked rats. Since the animals had never previously experienced the open door between the two compartments this behavior could not have been previously learned. It has been suggested that such behavior is due to a conditioned emotional response arising from an aversive Pavlovian association (McAllister & McAllister, 1995; Mowrer, 1947; Rescorla & Solomon, 1967) between cues in the paired compartment and the shock. According to this idea, the presence of a conditioned aversive internal state produces a tendency to avoid the conditioned cues that produce the state (see the description of the behavior of the shocked rats, above). Active avoidance of these conditioned cues reduces or eliminates the aversive state, reinforcing the behavior (Miller, 1948). This explanation leads to the inference of an aversive affective state from the observation of avoidance.

The present results suggest that memory modulation can lead to the inference of an aversive internal state in the same way as freezing and avoidance (Bindra & Anchel, 1963; Blanchard et al., 1968; Bolles & Collier, 1976; Davis, 1997; Fanselow, 1980; Fendt & Fanselow, 1999; Kiernan & Westbrook, 1993; LeDoux, 2000; McAllister & McAllister, 1995; Miller, 1948; Mowrer, 1947; Mowrer & Lamoreaux, 1946; Rescorla & Solomon, 1967). The fact that the relationship among these measures is weak or non-existent (see Figures 3A - C) may suggest that other factors also influence their occurrence. In particular, as shown here, freezing, avoidance, and activity are influenced by the configuration of the apparatus. These latter behaviors may therefore be "promoted" or facilitated by an internal aversive state, but as they are also influenced by other factors, they can be taken only as rough estimates of the existence of this state. The memory modulation response may be a more direct function of the internal state but, as it is ultimately measured by its effects on a learned behavior, it provides a similarly inexact estimate of the amplitude of an internal state.

EXPERIMENT 2

Amygdala lesions reduce or eliminate a variety of behaviors used to infer the presence of conditioned aversive states (Amorapanth et al., 2000; Antoniadis & McDonald, 2000; Davis, 2000; Fendt, 2001; Fendt & Fanselow, 1999; Holahan & White, 2002a; Killcross et al., 1997; LeDoux et al., 1988; Lee et al., 2001). These findings have led several authors to suggest

that the amygdala mediates the aversive Pavlovian associations (Fanselow & LeDoux, 1999; Fendt & Fanselow, 1999; LeDoux, 2000) that produce these states, and/or the states themselves (Davis, 2000; Fanselow, 1984; LeDoux, 2000).

The involvement of the amygdala in the three behaviors measured in the present experiment was examined by giving conditioned rats 2 training trials on the maze, administering intra-amygdala injections of muscimol or saline, and placing them in the shuttle-box apparatus with the door open. It was previously reported that electrolytic CeA or MeA lesions blocked memory modulation produced by a similar form of exposure to aversive conditioned stimuli (Holahan & White, 2002a). The absence of modulation in that study could have been due to impairment of acquisition or expression of the aversive association (Campeau et al., 1992; Fanselow & Kim, 1994; Miserendino et al., 1990) or to impaired memory for the modulated learning task. In Experiment 2 the $GABA_A$ agonist muscimol was used to inactivate the amygdala temporarily during posttraining exposure to the shuttle box. As the rats were in a normal state during the other parts of the experiment effects on modulation could be attributed to deficits in expression of the inferred internal conditioned modulatory response.

Intra-amygdala injections of muscimol have been shown to reduce freezing in the presence of an aversive CS (Helmstetter & Bellgowan, 1994; Muller et al., 1997; Wilensky et al., 1999; 2000) and to impair continuous multiple inhibitory avoidance (Coleman-Mesches & McGaugh, 1995), demonstrating their effectiveness in aversive conditioning procedures.

Materials and Methods

Subjects

Subjects were 34 rats as described in Experiment 1.

Surgery

All rats were food-deprived for 24 hours before surgery. They were anaesthetized with sodium pentobarbital (Somnotol, 65 mg/kg i.p.) and given 0.2 ml atropine sulphate s.c. Using standard stereotaxic techniques with the tooth bar set at - 3.5 mm (Paxinos & Watson, 1998), guide cannulas (26 ga; cut to 11 mm) were implanted at coordinates (in mm from bregma and skull) AP - 2.5, ML ± 4.2, DV - 6.0. Stylets were placed in the cannulas. Following surgery each rat was given 0.3 ml penicillin (300,000 u) and placed into a heated holding cage. After

recovery from anaesthesia the rats were given subcutaneous injections of 0.01 ml dipyrone hydrochloride and allowed to recover for one week before the behavioral procedure began.

Procedure

All apparatus was identical to that used in Experiment 1. The handling and food deprivation schedule were the same as described for Experiment 1 except that on the third handling day, each rat was given a bilateral intra-amygdala injection of physiological saline (1.0 μl/ side over 3 min). The stylets were removed from the guide cannulas and replaced with 32 ga injectors connected via plastic tubing to a minipump. The injectors extended 1.5 mm beyond the guide cannulas. The experimenter held each rat during the injection. After the injection the stylets were replaced and the rats were returned to their home cages.

The procedure was similar to that used for group 30Sh in Experiment 1. Preexposure to Box C and the shuttle-box were as described in Experiment 1. Twenty four hours later, 14 rats were shocked and 6 rats were not shocked in their paired compartments. Freezing was scored during this time. On the following day all rats were placed into their unpaired compartments with no shock.

Twenty four hours later the rats were trained on the radial maze CCP with 2 training trials. They were then placed into their home cages for 5 min. Seven shocked rats (Sh-mus) and 6 non-shocked rats (NSh-mus) were removed from their cages and given bilateral intra-amygdala injections of muscimol hydrobromide (0.44 nmol/ 0.5 μl; 1 μl/ side over 3 min with 2 min for post injection diffusion). The 7 remaining shocked rats (Sh-sal) received saline vehicle injections (same volume and rate as groups Sh-mus and Nsh-mus). After the injections, the rats were put back in their home cages. Fifteen min later they were placed into their paired compartment in the shuttle box with the door between the compartments open. The delay between CCP training and posttraining exposure to the conditioned cues was 25 - 30 min.

Two additional groups that were not given aversive conditioning were given 4 CCP training trials and then replaced in their home cages for 5 min. Each rat was then removed from its cage and given intra-amygdala injections of muscimol (n = 7) or saline (n = 7) using the injection parameters described above. They were then returned to their home cages.

The rats in all 5 groups were tested on the CCP task 24 hours after the posttraining

treatments, as described in Experiment 1. All statistical analyses were as described in Experiment 1.

Histology

Upon completion of testing, the rats were given an overdose of 30% chloral hydrate i.p. and perfused transcardially with 0.9% saline followed by 10% formal saline. The brains were removed and stored in 10% formal saline for approximately one week. They were then frozen with dry ice and 30 μm sections were cut through the injector tracks on a cryostat. The sections were mounted on glass slides and stained with formal thionin (Donovick, 1974). Grayscale digital brain images were captured using Scion Image and contrast enhanced and sharpened using Corel Photo-Paint v.10.

Results

Figure 4 illustrates the injection sites. The tips of the injector cannulas were typically located dorsal to the central nucleus of the amygdala and did not produce any visible gliosis or damage to neurons in the central or lateral nuclei. The volume and dose of drug used has been shown to reduce c-Fos labeling within the central, basomedial and, basolateral nuclei as well as in ventral parts of the lateral and dorsal medial nuclei (Holahan & White, 2002b). There were no consistent differences in the placements of the guide cannulas among the groups.

Figure 5A shows that there was a significant preference for the food-paired radial maze arm in group Sh-Sal ($F(1,29) = 29.90$, $p < 0.001$) but no such difference in group Sh-mus ($F(1,29) = 1.53$) or group NSh-mus ($F(1,29) < 1.0$). This shows that posttraining exposure to the conditioned cues enhanced retention of the CCP (as in Experiment 1), and that intra-amygdala muscimol eliminated this effect.

Figure 5B shows that posttraining inactivation of the amygdala with muscimol did not affect the expression of the unmodulated CCP after 4 training trials. There were significant preferences for the paired arms in both the muscimol group ($F(1,12) = 8.24$, $p < 0.05$) and the saline control group ($F(1,12) = 23.73$, $p < 0.001$). Neither the interaction from this analysis ($F(1,12) < 1.0$) nor a t-test comparing the differences between the paired and unpaired arms for the two groups ($t(12) < 1.0$) were significant. This indicates that both groups showed similar conditioned preferences for the paired arm.

Table 1 shows percent freezing during the shock-training session and the first bout in the paired compartment during the posttraining treatment. A main effect of group ($F(2,17) = 15.06$, $p < 0.001$) confirmed that both shocked groups (Sh-Sal and Sh-Mus) froze more than the no shock group during the training session (t values > 6.0, $p < 0.01$). A main effect of day ($F(1,2) = 66.00$, $p < 0.001$) showed that both groups Sh-Sal and Sh-Mus froze less during the open door test than during shock training (t values > 3.0, $p < 0.01$). A significant interaction between group and day ($F(2,17) = 9.99$, $p < 0.01$) showed that the decrease was larger in group Sh-Mus than in group Sh-Sal. During the first bout in the paired compartment, group Sh-Sal froze more than both groups Sh-Mus and NSh-Mus (t values > 2.0, $p < 0.05$).

Analysis of the avoidance ratios (Figure 6A) revealed significant overall group differences ($F(2,17) = 10.78$, $p < 0.01$). Group Sh-Sal spent more time in the unpaired compartment than groups Sh-Mus and NSh-Mus (t values > 7.5, p values < 0.01 for both comparisons). Groups Sh-mus and NSh-mus were not significantly different ($t(11) = 1.30$).

Analysis of the crossover rates (Figure 6B) revealed significant overall group differences ($F(2,17) = 7.64$, $p < 0.01$). Group Sh-Sal showed a lower crossover rate than groups Sh-Mus and NSh-Mus (t values > 3.0, p values < 0.01 for both comparisons). There was no significant difference between Groups Sh-Mus and NSh-Mus ($t(11) < 1.0$).

Discussion of Experiment 2

The elimination of conditioned memory modulation by amygdala inactivation is consistent with a previous finding (Holahan & White, 2002a) that CeA or MeA lesions blocked the memory enhancement produced by posttraining exposure to shock conditioned contextual and tone cues. The present finding that a similar effect was produced by temporary inactivation of the amygdala prior to the posttraining treatment is consistent with the hypothesis that a functional amygdala is required for expression of a conditioned response that modulates memory.

The hypothesis that the observed impairment was specific to memory modulation is supported by the finding that posttraining intra-amygdala injections of muscimol following 4 CCP training trials did not impair expression of the CCP. In a previous study using the same training procedure (in which rats are moved between the paired and unpaired arms by the experimenter; (White & Ouellet, 1997), neither fimbria-fornix nor LA lesions blocked the

expression of the CCP. The authors suggested that this version of the CCP can be learned in two different ways by two independent memory systems. The present results show that an intact amygdala during the posttraining period is not required for acquisition of this form of CCP learning provided sufficient training is given. The results also suggest that a CCP can be acquired by normal rats with less training followed by exposure to conditioned aversive cues. This learned behavior may be due to amygdala-mediated modulation of a hippocampus-based memory (White & McDonald, 2002; White & Ouellet, 1997).

The central importance of the amygdala to the memory modulating effects of unconditioned posttraining treatments has been well documented (McGaugh, 2000; 2002; McGaugh et al., 2000; Packard & Cahill, 2001). Combined lesions of the BLA/CeA (Ammassari-Teule et al., 1991) or lesions restricted to the BLA or MeA (Roozendaal & McGaugh, 1996) block unconditioned memory modulation. Pharmacological manipulations of the BLA (Da Cunha et al., 1999; Power et al., 2000; Quirarte et al., 1997) or CeA (Introini-Collison et al., 1996) also block unconditioned memory modulation. These data indicate that the CeA, BLA, or MeA may participate in mediating unconditioned modulation in various conditions.

In Holahan & White (2002a) electrolytic lesions confined to the CeA or MeA but not to the LA or BLA blocked the memory improving effect of posttraining exposure to shock-paired cues. Although the present findings also indicate that the amygdala mediates the memory modulating effect of conditioned aversive posttraining treatments, the injection volume used does not allow any conclusions about which specific amygdala subnuclei might mediate expression of conditioned memory modulation.

Increased freezing and avoidance, and lower crossover rates in the shocked rats were also eliminated by intra-amygdala muscimol. The effect on freezing is consistent with previous findings that injections of muscimol into the amygdala block increased freezing during exposure to shock conditioned cues (Helmstetter & Bellgowan, 1994; Muller et al., 1997). In those studies freezing was blocked with a 10-fold higher dose of muscimol and more lateral injection placements than were used in the present study. Other previous studies (eg, Amorapanth et al., 2000; Goosens & Maren, 2001; Holahan & White, 2002a; Killcross et al., 1997; Nader et al., 2001) show that a variety of restricted amygdala lesions block enhanced

freezing.

The rats in group Sh-Sal froze for an average of about 20% of their first bout in the paired compartment and were largely immobile (mainly head and body movements directed at the open door, as described in Experiment 1) for the remainder of the bout. The rats in group Sh-Mus did not freeze according to the strict definition, nor did they exhibit the slow movements directed at the door. Instead, they tended to move around normally and escape quickly. This can be seen from the similarity between the durations of the first paired bouts for groups Sh-Mus (12.9 ± 2.2; mean ± SEM) and Sh-Sal (10.2 ± 1.7). Elevated freezing or immobility would have increased bout duration.

The mean crossover rates in groups Sh-Mus and NSh-Sal were similar, and both were higher than the rate for the Sh-Sal group. The crossover rates for groups Sh-Mus and NSh-Sal were similar to that for the no-shock group in Experiment 1 (t-test comparison not significant). This lack of a detectable effect of muscimol on locomotor activity is consistent with previous reports that amygdala lesions do not change general activity levels (Decker et al., 1995; Goldstein, 1968; Maren, 1998).

The elimination of both freezing and immobility and the normalization of the crossover rate by intra-amygdala muscimol injections prior to exposure to the conditioned cues is consistent with the hypothesis that a functional amygdala is essential for the normal expression of these behaviors.

Intra-amygdala injection of muscimol prior to the test also blocked avoidance, a behavior that is incompatible with freezing and immobility. This means that the effects of amygdala inactivation could not have been due to an inability of the rats to perform any unconditioned or conditioned behavior. Rather, the results lead to the conclusion that the experimental treatment blocked a conditioned response that could have produced all of these behaviors in normal rats. This conditioned response could have been an internal aversive state. As already described, conditioned avoidance has long been thought to be based on such an internal state (Miller, 1948; Mowrer, 1947; Rescorla & Solomon, 1967).

General Discussion

The two main findings of this study concern the effects of aversive Pavlovian conditioning. First, conditioned memory modulation was confirmed as one effect of such

conditioning. Although it is not a directly observable response, conditioned memory modulation can be studied in parallel with observable behaviors such as freezing and avoidance. Second, normal activity of neurons in the amygdala is required for all three of these behaviors. The findings suggest that when normal conditioned rats are exposed to an aversive CS neurons in the amygdala are activated producing an internal conditioned aversive sate ("fear"). The behaviors measured (improved memory, freezing and avoidance) are produced or promoted by this state.

What is the Conditioned Response?

In the present experiment, as in other aversive Pavlovian conditioning procedures, exposure to initially neutral cues in the paired compartment (CS) preceded the occurrence of a shock (US). Some freezing, but no avoidance (the door was closed) occurred during this session. When subsequently exposed to the CS alone, both freezing and avoidance were observed.

Normally, the shock US produces only a small amount of freezing (< 10% of the observation time; see Blanchard & Blanchard, 1969 Fig 1, p 371; Bolles & Collier, 1976 Fig 1, p 7; Fanselow, 1980 Table 1, p 179) while exposure to the aversive CS produces much more freezing (> 50% of the observation time; Fanselow, 1982; 1984; Fanselow et al., 1994; Kiernan et al., 1995; Maren, 2001; Sacchetti et al., 1999). This difference suggests that freezing itself may not be the CR. Rather, as suggested by others (Davis, 1997; Fanselow, 1984; LeDoux, 1998; LeDoux, 2000; Maren, 2001), the CR may be an unobservable internal aversive state and the observed freezing may be a behavior that is facilitated or promoted in the presence of that state when escape behaviors are either not available or when available behaviors have not been learned. Freezing could also result from an additional conditioning process (i.e., conditioning of motor representations) that is parallel to the one mediating the affective state. This suggests that freezing could occur in the absence of an aversive affective state.

As already discussed, the avoidance behavior observed could not have been the CR; this behavior is thought to be acquired as an instrumental response that resulted in the reduction of an aversive internal CR (Miller, 1948; Mowrer, 1947; Mowrer & Lamoreaux, 1946; Rescorla, 1968; Rescorla & Solomon, 1967; Siddle & Bond, 1988).

Memory modulation is produced by exposure to both unconditioned (Holahan & White, 2002a; Jodar et al., 1996; White & Legree, 1984) and conditioned (Holahan & White, 2002a); present study) aversive stimuli. It is possible (even likely) that these modulation effects are produced by one or more of several autonomic, hormonal and neural responses that constitute the unconditioned and conditioned internal states produced by exposure to the US or CS in these experiments.

Amygdala and expression of the aversive CR

Evidence that muscimol inactivates cell bodies without affecting fibers of passage (Lomber, 1999; Malpeli, 1999; Martin & Ghez, 1999) suggests that the elimination of freezing, avoidance and conditioned memory modulation by intra-amygdala muscimol injections was due specifically to diminished activity of neurons intrinsic to the amygdala. This conclusion is consistent with the idea that normal activity of neurons in the amygdala is required for expression of the CR, a set of neural and hormonal responses constituting an internal affective state and thought to be the basis of the observed behaviors.

If normal activity in amygdala neurons is essential for expression of the CR in aversive Pavlovian conditioning, there are several possibilities as to the nature of the information this activity represents. The design of the experiment allows the elimination of at least some of these possibilities. Both freezing and avoidance were measured during the same session of exposure to the CS when the door between the two compartments was open for the first time. As freezing tends to interfere with the expression of avoidance (Anisman, 1973; Anisman & Waller, 1972; 1973), the simultaneous measurement of these incompatible behaviors controls for the possibility that the intra-amygdala muscimol affected representations of motor elements of the aversive Pavlovian conditioning. If the rats' ability to freeze had been affected, they should still have exhibited avoidance. Similarly, if their ability to perform the avoidance behavior had been affected, they should still have frozen. The fact that both of these behaviors were reduced while the rats remained normally active suggests that the injections did not affect the rats' ability to perform either of these behaviors.

These conclusions are consistent with other findings showing that lesions of the amygdala reduce freezing (Amorapanth et al., 2000; Holahan & White, 2002a; Kim et al., 1993; Maren, 1998;1999; Nader et al., 2001), avoidance (Ambrogi Lorenzini et al., 1991; Amorapanth et

al., 2000; Antoniadis & McDonald, 2000; Holahan & White, 2002a; Jellestad & Cabrera, 1986; Killcross et al., 1997; Smith et al., 2001), and other behaviors elicited by aversive CSs (Campeau et al., 1992; Fendt, 2001; Hitchcock & Davis, 1991; Kim & Davis, 1993; Lee et al., 2001; Miserendino et al., 1990). These authors hypothesized that lesions of the amygdala interfered with associative information underlying the expression of the observed behaviors (freezing, avoidance, fear-potentiated startle). The present results are consistent with this hypothesis.

Memory modulation may be of special importance for understanding the nature and properties of the hypothesized conditioned internal state. To the extent that conditioned modulation is due to some component(s) of the same amygdala-mediated internal state that promotes freezing and avoidance, information about specific internal responses that produce modulation may reveal some of the properties of this state. For example, aversive CSs elicit norepinephrine (Korte et al., 1992a; Korte et al., 1992b) and glucose (Surwit et al., 1985) release. Posttraining norepinephrine modulates memory via its promotion of glucose release (Gold et al., 1986; Gold, 1995; Gold & van Buskirk, 1975; Hall & Gold, 1986). Accordingly, elevated norepinephrine and glucose are possible constituents of the aversive internal state.

Memory modulation is known to be an amygdala-mediated response that affects memories in other parts of the brain (McGaugh et al., 1996; McGaugh, 2002; Packard et al., 1994; Packard & Cahill, 2001; Packard & Teather, 1998). The present findings suggest that a memory for the conditioned aversive response, one effect of which is modulation, must exist somewhere in the brain. If this memory is the conditioned Pavlovian association that is thought to underlie freezing and avoidance the evidence discussed suggests that it is probably in a neural system that includes the amygdala (White & McDonald, 2002). It is also possible that these associations are in some other neural system that can influence the amygdala to produce the internal conditioned response. These alternatives will require further investigation.

In summary, the present results provide a further demonstration that posttraining exposure to shock-conditioned aversive cues can modulate memory. Exposure to the same aversive cues also promoted the expression of freezing and avoidance, two observable behaviors that have competing topographies. Since intra-amygdala injections of muscimol blocked the

expression of two incompatible behaviors these effects could not have been due to an inability to perform the behaviors measured. The same injections of muscimol also blocked conditioned memory modulation. Together, these effects suggest that the amygdala system produces a set of unobservable conditioned responses that comprise a conditioned affective state. The modulation response may consist of some component of the internal aversive affective state.

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References

Ambrogi Lorenzini,C., Bucherelli,C., Giachetti,A., Mugnai,L., & Tassoni,G. (1991). Effects of nucleus basolateralis amygdalae neurotoxic lesions on aversive conditioning in the rat. *Physiology and Behavior, 49*, 765-770.

Ammassari-Teule,M., Pavone,F., Castellano,C., & McGaugh,J.L. (1991). Amygdala and dorsal hippocampus lesions block the effects of GABAergic drugs on memory storage. *Brain Research, 551*, 104-109.

Amorapanth,P., LeDoux,J.E., & Nader,K. (2000). Different lateral amygdala outputs mediate reactions and actions elicited by a fear-arousing stimulus. *Nature Neuroscience, 3*, 74-79.

Anisman,H. (1973). Effects of pretraining compatible and incompatible responses on subsequent one-way and shuttle-avoidance performance in rats. *Journal of Comparative and Physiological Psychology, 82*, 95-104.

Anisman,H. & Waller,T.G. (1972). Facilitative and disruptive effects of prior exposure to shock on subsequent avoidance performance. *Journal of Comparative and Physiological Psychology, 78*, 113-122.

Anisman,H. & Waller,T.G. (1973). Effects of inescapable shock on subsequent avoidance performance: role of response repertoire changes. *Behavioral Biology, 9*, 331-355.

Antoniadis,E.A. & McDonald,R.J. (1999). Discriminative fear conditioning to context expressed by multiple measures of fear in the rat. *Behavioural Brain Research, 101*, 1-13.

Antoniadis,E.A. & McDonald,R.J. (2000). Amygdala, hippocampus and discriminative fear conditioning to context. *Behavioural Brain Research, 108*, 1-19.

Barnett,S.A. (1963). Movement in the living space. *The rat: A study in behavior* (pp. 15 - 33). Chicago: Aldine Publishing Company.

Baron,A. (1964). Suppression of exploratory behavior by aversive stimulation. *Journal of Comparative and Physiological Psychology, 57*, 299-301.

Bindra,D. & Anchel,H. (1963). Immobility as an avoidance response, and its disruption by drugs. *Journal of the Experimental Analysis of Behavior, 6*, 213-218.

Blanchard,D.C. & Blanchard,R.J. (1972). Innate and conditioned reactions to threat in rats with amygdaloid lesions. *Journal of Comparative and Physiological Psychology, 81*, 281-290.

Blanchard,R.J. & Blanchard,C. (1968). Escape and avoidance responses to a fear eliciting situation. *Psychonomic Sciences, 13*, 19-20.

Blanchard,R.J. & Blanchard,C. (1969). Crouching as an index of fear. *Journal of*

Comparative and Physiological Psychology, 67, 370-375.

Blanchard,R.J. & Blanchard,D.C. (1970a). Dual mechanisms in passive avoidance: I. *Psychonomic Sciences, 19*, 1-2.

Blanchard,R.J. & Blanchard,D.C. (1970b). Dual mechanisms in passive avoidance: II. *Psychonomic Sciences, 19*, 3-4.

Blanchard,R.J. & Blanchard,D.C. (1971). *Defensive reactions in the albino rat. Learning and Motivation, 2*, 351-362.

Blanchard,R.J., Dielman,T.E., & Blanchard,D.C. (1968). Prolonged aftereffects of a single foot shock. *Psychonomic Sciences, 10*, 327-328.

Bolles,R.C. & Collier,A.C. (1976). The effect of predictive cues on freezing in rats. *Animal Learning and Behavior, 4*, 6-8.

Bolles,R.C. (1970). Species-specific defensive reactions and avoidance learning. *Psychological Review, 77*, 32-48.

Brener,J. & Goesling,W.J. (1970). Avoidance conditioning of activity and immobility in rats. *Journal of Comparative and Physiological Psychology, 70*, 276-280.

Brown,J.S. & Jacobs,A. (1949). The role of fear in the motivation and acquisition of responses. *The Journal of Experimental Psychology, 39*, 747-759.

Cahill,L. (2000). Modulation of long-term memory storage in humans by emotional arousal: adrenergic activation and the amygdala. In J. P. Aggleton (ed.), *The Amygdala: A Functional Analysis* (2nd ed), (pp. 425 - 445). Oxford: Oxford University Press.

Cahill,L. & McGaugh,J.L. (1991). NMDA-induced lesions of the amygdaloid complex block the retention-enhancing effect of posttraining epinephrine. *Psychobiology, 19*, 206-210.

Cahill,L. & McGaugh,J.L. (1996). Modulation of memory storage. *Current Opinion in Neurobiology, 6*, 237-242.

Cahill,L., McGaugh,J.L., & Weinberger,N.M. (2001). The neurobiology of learning and memory: some reminders to remember. *Trends in Neurosciences, 24*, 578-581.

Cahill,L., Vazdarjanova,A., & Setlow,B. (2000). The basolateral complex is involved with, but is not necessary for, rapid acquisition of Pavlovian 'fear conditioning'. *European Journal of Neuroscience, 12*, 3044-3050.

Cahill,L., Weinberger,N.M., Roozendaal,B., & McGaugh,J.L. (1999). Is the amygdala a locus of "conditioned fear"? Some questions and caveats. *Neuron, 23*, 327-328.

Campbell,B.A. & Campbell,E.H. (1962). Retention and extinction of learned fear in infant and adult rats. Journal of Comparative and Physiological Psychology *55*, 1-8.

Campeau,S., Miserendino,M.J.D., & Davis,M. (1992). Intra-amygdala infusion of the *N*-Methyl-D-Aspartate receptor antagonist AP5 blocks acquisition but not expression of fear-potentiated startle to an auditory conditioned stimulus. *Behavioral Neuroscience, 106*, 569-574.

Claus,H.-J. & Bindra,D. (1960). Reactions to novelty and stimulus-change induced response decrement. *Canadian Journal of Psychology, 14*, 101-110.

Coleman-Mesches,K. & McGaugh,J.L. (1995). Muscimol injected into the right or left amygdaloid complex differentially affects retention performance following aversively motivated training. *Brain Research, 676*, 183-188.

Da Cunha,C., Roozendaal,B., Vazdarjanova,A., & McGaugh,J.L. (1999). Microinfusions of flumazenil into the basolateral but not the central nucleus of the amygdala enhance memory consolidation in rats. *Neurobiology of Learning and Memory, 72*, 1-7.

Davis,M. (1997). Neurobiology of fear responses: the role of the amygdala. *Journal of Neuropsychiatry and Clinical Neurosciences, 9*, 382-402.

Davis,M. (2000). The role of the amygdala in conditioned and unconditioned fear and anxiety. In J. P. Aggleton (ed.), *The Amygdala: A Functional Analysis* (2nd ed), (pp. 213 - 287). Oxford: Oxford University Press.

Decker,M.W., Curzon,P., & Brioni,J.D. (1995). Influence of separate and combined septal and amygdala lesions on memory, acoustic startle, anxiety, and locomotor activity in rats. *Neurobiology of Learning and Memory, 64*, 156-168.

Donovick,P.J. (1974). A metachromatic stain for neural tissue. *Stain Technology, 49*, 49-51.

Fanselow,M.S. (1980). Conditional and unconditional components of postshock freezing. *Pavlovian Journal of Biological Sciences, 15*, 177-182.

Fanselow,M.S. (1982). The postshock activity burst. *Animal Learning and Behavior, 10*, 448-454.

Fanselow,M.S. (1984). What is conditioned fear? *Trends in Neurosciences, 7*, 460-462.

Fanselow,M.S. & Kim,J.J. (1994). Acquisition of contextual Pavlovian fear conditioning is blocked by application of an NMDA receptor antagonist D,L-2-Amino-5-Phosphonovaleric Acid to the basolateral amygdala. *Behavioral Neuroscience, 108*, 210-212.

Fanselow,M.S., Landeira-Fernandez,J., DeCola,J.P., & Kim,J.J. (1994). The immediate-shock deficit and postshock analgesia: implications for the relationship between the analgesic CR and UR. *Animal Learning and Behavior, 22*, 72-76.

Fanselow,M.S. & LeDoux,J.E. (1999). Why we think plasticity underlying Pavlovian fear

conditioning occurs in the basolateral amygdala. *Neuron, 23*, 229-232.

Fendt,M. (2001). Injections of the NMDA receptor antagonist aminophosphonopentanoic acid into the lateral nucleus of the amygdala block the expression of fear-potentiated startle and freezing. *The Journal of Neuroscience, 21*, 4111-4115.

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

Gaston,M.G. & Freed,L. (1969). Effect of amygdaloid lesions in a fear conditioning situation not involving instrumental learning. *Psychonomic Sciences, 16*, 55-56.

Gold,P.E. (1995). Role of glucose in regulating the brain and cognition. *American Journal of Clinical Nutrition, 61(suppl)*, 987S-995S.

Gold,P.E. & McGaugh,J.L. (1975). A single-trace, two-process view of memory storage processes. In D. Deutsch and J. A. Deutsch (Eds), *Short-Term Memory* (pp. 355 - 378). New York: Academic Press.

Gold,P.E. & van Buskirk,R.B. (1975). Facilitation of time-dependent memory processes with posttrial epinephrine injection. *Behavioral and Neural Biology, 31*, 247-260.

Gold,P.E., Vogt,J., & Hall,J.L. (1986). Glucose effects on memory: behavioral and pharmacological characteristics. *Behavioral and Neural Biology, 46*, 145-155.

Goldstein,M.L. (1968). Effects of lesions of the amygdaloid complex on peripheral shock thresholds and activity in the hooded rat. *The Journal of General Psychology, 79*, 59-74.

Goosens,K.A. & Maren,S. (2001). Contextual and auditory fear conditioning are mediated by the lateral, basal, and central amygdaloid nuclei in rats. *Learning and Memory, 8*, 148-155.

Hall,J.L. & Gold,P.E. (1986). The effects of training, epinephrine, and glucose injections on plasma glucose levels in rats. *Behavioral and Neural Biology, 46*, 156-167.

Helmstetter,F.J. & Bellgowan,P.S. (1994). Effects of muscimol applied to the basolateral amygdala on acquisition and expression of contextual fear conditioning in rats. *Behavioral Neuroscience, 108*, 1005-1009.

Hitchcock,J.M. & Davis,M. (1991). Efferent pathway of the amygdala involved in conditioned fear as measured with the fear-potentiated startle paradigm. *Behavioral Neuroscience, 105*, 826-842.

Holahan,M.R. & White,N.M. (1999). Exposure to an aversive conditioned context and c-Fos labeling in the amygdala. *Society for Neuroscience Abstracts, 25*, 647.16.

Holahan,M.R. & White,N.M. (2001). Two-process learning theory and multiple memory

systems. *Society for Neuroscience Abstracts, 27*, 743.2.

Holahan,M.R. & White,N.M. (2002a). Conditioned memory modulation, freezing, and avoidance as measures of amygdala-mediated conditioned fear. *Neurobiology of Learning and Memory, 77*, 250-275.

Holahan,M.R. & White,N.M. (2000b) Effect of muscimol inactivation of the basolateral or central amygdala on shock-conditioned responses. *Annals of the New York Academy of Sciences*. (In press).

Introini-Collison,I., Dalmaz,C., & McGaugh,J.L. (1996). Amygdala β-noradrenergic influences on memory storage involve cholinergic activation. *Neurobiology of Learning and Memory, 65*, 57-64.

Jellestad,F.K. & Cabrera,I.G. (1986). Exploration and avoidance learning after ibotenic acid and radio frequency lesions in the rat amygdala. *Behavioral and Neural Biology, 46*, 196-215.

Jodar,L., Takahashi,M., & Kaneto,H. (1996). FS stress induces long-lasting memory facilitation: involvement of cholinergic pathways. *Pharmacology, Biochemistry, and Behavior, 53*, 735-740.

Kiernan,M.J. & Westbrook,R.F. (1993). Effects of exposure to a to-be-shocked environment upon the rat's freezing response: evidence for facilitation, latent inhibition, and perceptual learning. *The Quarterly Journal of Experimental Psychology, 46B*, 271-288.

Kiernan,M.J., Westbrook,R.F., & Cranney,J. (1995). Immediate shock, passive avoidance, and potentiated startle: implications for the unconditioned response to shock. *Animal Learning and Behavior, 23*, 22-30.

Killcross,S., Robbins,T.W., & Everitt,B.J. (1997). Different types of fear-conditioned behaviour mediated by separate nuclei within the amygdala. *Nature, 388*, 377-380.

Kim,J.J., Rison,R.A., & Fanselow,M.S. (1993). Effects of amygdala, hippocampus, and periacqueductal gray lesions on short- and long-term contextual fear. *Behavioral Neuroscience, 107*, 1093-1098.

Kim,M. & Davis,M. (1993). Lack of temporal gradient of retrograde amnesia in rats with amygdala lesions assessed with fear-potentiated startle paradigm. *Behavioral Neuroscience, 107*, 1088-1092.

Kirk,R.E. (1969). *Experimental design: procedures for the behavioral sciences*. Belmont, CA: Wadsworth.

Korte,S.M., Bouws,G.A.H., Koolhaas,J.M., & Bohus,B. (1992a). Neuroendocrine and behavioral responses during conditioned active and passive behavior in the defensive burying/probe avoidance paradigm: effects of ipsapirone. *Physiology and Behavior, 52*,

355-361.

Korte,S.M., Buwalda,B., Bouws,G.A.H., Koolhaas,J.M., Maes,F.W., & Bohus,B. (1992b). Conditioned neuroendocrine and cardiovascular stress responsiveness accompanying behavioral passivity and activity in aged and in young rats. *Physiology and Behavior, 51*, 815-822.

Kumar,R. (1970). Effects of fear on exploratory behaviour in rats. *Quarterly Journal of Experimental Psychology, 22*, 205-214.

LeDoux,J. (1998). Fear and the brain: where have we been, and where are we going? *Biological Psychiatry, 44*, 1229-1238.

LeDoux,J. (2000). The amygdala and emotion: a view through fear. In J. P. Aggleton (ed.), *The Amygdala: A Functional Analysis* (2nd ed), (pp. 289 - 310). Oxford: Oxford University Press.

LeDoux,J.E. (1996). Emotional networks and motor control: a fearful view. In G. Holstege, R. Bandler, and C. B. Saper (Eds), *Progress in Brain Research* (pp. 437 - 446). Elsevier Science.

LeDoux,J.E., Iwata,J., Cicchetti,P., & Reiss,D.J. (1988). Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlate of conditioned fear. *The Journal of Neuroscience, 8*, 2517-2529.

Lee,H.J., Choi,J.-S., Brown,T.H., & Kim,J.J. (2001). Amygdalar NMDA receptors are critical for the expression of multiple conditioned fear responses. *The Journal of Neuroscience, 21*, 4116-4124.

Lee,Y., Walker,D.L., & Davis,M. (1996). Lack of a temporal gradient of retrograde amnesia following NMDA-induced lesions of the basolateral amygdala assessed with the fear-potentiated startle paradigm. *Behavioral Neuroscience, 110*, 836-839.

Lomber,S.G. (1999). The advantages and limitations of permanent or reversible deactivation techniques in the assessment of neuronal function. *Journal of Neuroscience Methods, 86*, 109-117.

Malpeli,J.G. (1999). Reversible inactivation of subcortical sites by drug injection. *Journal of Neuroscience Methods, 86*, 119-128.

Maren,S. (1998). Overtraining does not mitigate contextual fear conditioning deficits produced by neurotoxic lesions of the basolateral amygdala. *The Journal of Neuroscience, 18*, 3088-3097.

Maren,S. (1999). Neurotoxic basolateral amygdala lesions impair learning and memory but not the performance of conditional fear in rats. *The Journal of Neuroscience, 19*, 8696-8703.

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

Maren,S., Aharonov,G., Stote,D.L., & Fanselow,M.S. (1996). *N*-Methyl-D-Aspartate receptors in the basolateral amygdala are required for both acquisition and expression of conditional fear in rats. *Behavioral Neuroscience, 110*, 1365-1374.

Martin,J.H. & Ghez,C. (1999). Pharmacological inactivation in the analysis of the central control of movement. *Journal of Neuroscience Methods, 86*, 145-159.

Maxwell,S.E. & Delaney,H.D. (1990). *Designing experiments and analyzing data* (pp. 170 - 206). Pacific Grove, CA: Brooks/Cole Publishing Co.

McAllister,W.R. & McAllister,D.E. (1962). Role of CS and of apparatus cues in the measurement of acquired fear. *Psychological Reports, 11*, 749-756.

McAllister,W.R. & McAllister,D.E. (1971). Behavioral measurement of conditioned fear. In F. R. Brush (Ed.) *Aversive Conditioning and Learning* (pp. 105 - 179). New York: Academic Press.

McAllister,W.R. & McAllister,D.E. (1995). Two-factor fear theory: implications for understanding anxiety-based clinical phenomena. In W. O'Donohue and L. Krasner (Eds.), *Theories of Behavior Therapy: Exploring Behavior Change* (pp. 145 - 171). Washington, D.C: American Psychological Association.

McDonald,R.J. & White,N.M. (1995a). Hippocampal and nonhippocampal contributions to place learning in rats. *Behavioral Neuroscience, 109*, 579-593.

McDonald,R.J. & White,N.M. (1995b). Information acquired by the hippocampus interferes with acquisition of the amygdala-based conditioned-cue preference in the rat. *Hippocampus, 5*, 189-197.

McGaugh,J.L. (1966). Time-dependent processes in memory storage. *Science, 153*, 1351-1358.

McGaugh,J.L. (2000). Memory - a century of consolidation. *Science, 287*, 248-251.

McGaugh,J.L. (2002). Memory consolidation and the amygdala: a systems perspective. *Trends in Neurosciences, 25*, 456-561.

McGaugh,J.L. & Cahill,L. (1997). Interaction of neuromodulatory systems in modulating memory storage. *Behavioural Brain Research, 83*, 31-38.

McGaugh,J.L., Cahill,L., & Roozendaal,B. (1996). Involvement of the amygdala in memory storage: interaction with other brain systems. *Proceedings of the National Academy of Sciences of the United States of America, 93*, 13508-13514.

McGaugh,J.L., Ferry,B., Vazdarjanova,A., & Roozendaal,B. (2000). Amygdala: role in modulation of memory storage. In J. P. Aggleton (ed.), *The Amygdala: A Functional Analysis* (2nd ed), (pp. 391 - 423). Oxford: Oxford University Press.

Miller,N.E. (1948). Studies of fear as an acquirable drive: I. Fear as motivation and fear-reduction as reinforcement in the learning of new responses. *Journal of Experimental Psychology, 38*, 89-101.

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

Mowrer,O.H. (1947). On the dual nature of learning -- A re-interpretation of "conditioning" and "problem-solving". *Harvard Educational Review, 17*, 102-148.

Mowrer,O.H. & Lamoreaux,R.R. (1946). Fear as an intervening variable in avoidance conditioning. *Journal of Comparative Psychology, 39*, 29-50.

Muller,J., Corodimas,K.P., Fridel,Z., & LeDoux,J.E. (1997). Functional inactivation of the lateral and basal nuclei of the amygdala by muscimol infusion prevents fear conditioning to an explicit conditioned stimulus and to contextual stimuli. *Behavioral Neuroscience, 111*, 683-691.

Nader,K., Majidishad,P., Amorapanth,P., & LeDoux,J.E. (2001). Damage to the lateral and central, but not other, amygdaloid nuclei prevents the acquisition of auditory fear conditioning. *Learning and Memory, 8*, 156-163.

Olton,D.S. (1973). Shock-motivated avoidance and the analysis of behavior. *Psychological Bulletin, 79*, 243-251.

Packard,M.G. & Cahill,L. (2001). Affective modulation of multiple memory systems. *Current Opinion in Neurobiology, 11*, 752-756.

Packard,M.G., Cahill,L., & McGaugh,J.L. (1994). Amygdala modulation of hippocampal-dependent and caudate nucleus-dependent memory processes. *Proceedings of the National Academy of Sciences of the United States of America, 91*, 8477-8481.

Packard,M.G. & Teather,L.A. (1998). Amygdala modulation of multiple memory systems: hippocampus and caudate-putamen. *Neurobiology of Learning and Memory, 69*, 163-203.

Paxinos,G. & Watson,C. (1998). *The rat brain atlas in stereotaxic coordinates* (4th ed.). San Diego, CA: Academic Press.

Phillips,R.G. & LeDoux,J.E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behavioral Neuroscience, 106*, 274-285.

Poremba,A. & Gabriel,M. (1997). Amygdalar lesions block discriminative avoidance learning and cingulothalamic training-induced neuronal plasticity in rabbits. *The Journal of Neuroscience, 17*, 5237-5244.

Power,A.E., Roozendaal,B., & McGaugh,J.L. (2000). Glucocorticoid enhancement of memory consolidation in the rat is blocked by muscarinic receptor antagonism in the basolateral amygdala. *European Journal of Neuroscience, 12*, 3481-3487.

Quirarte,G.L., Roozendaal,B., & McGaugh,J.L. (1997). Glucocorticoid enhancement of memory storage involves noradrenergic activation in the basolateral amygdala. *Proceedings of the National Academy of Sciences of the United States of America, 94*, 14048-14053.

Rescorla,R.A. (1968). Pavlovian conditioned fear in Sidman avoidance learning. *Journal of Comparative and Physiological Psychology, 65*, 55-60.

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

Roozendaal,B. & McGaugh,J.L. (1996). Amygdaloid nuclei lesions differentially affect glucocorticoid-induced memory enhancement in an inhibitory avoidance task. *Neurobiology of Learning and Memory, 65*, 1-8.

Sacchetti,B., Lorenzini,C.A., Baldi,E., Tassoni,G., & Bucherelli,C. (1999). Auditory thalamus, dorsal hippocampus, basolateral amygdala, and perirhinal cortex role in the consolidation of conditioned freezing to context and to acoustic conditioned stimulus in the rat. *The Journal of Neuroscience, 19*, 9570-9578.

Save,E., Poucet,B., Foreman,N., & Buhot,M.-C. (1992). Object exploration and reactions to spatial and nonspatial changes in hooded rats following damage to parietal cortex or hippocampal formation. *Behavioral Neuroscience, 106*, 447-456.

Selden,N.R.W., Everitt,B.J., Jarrard,L.E., & Robbins,T.W. (1991). Complementary roles for the amygdala and hippocampus in aversive conditioning to explicit and contextual cues. *Neuroscience, 42*, 335-350.

Siddle,D.A.T. & Bond,N.W. (1988). Avoidance learning, Pavlovian conditioning, and the development of phobias. *Biological Psychology, 27*, 167-183.

Smith,D.M., Monteverde,J., Schwartz,E., Freeman,J.H., & Gabriel,M. (2001). Lesions of the central nucleus of the amygdala: discriminative avoidance learning, discriminative approach learning, and cingulothalamic training-induced neuronal activity. *Neurobiology of Learning and Memory, 76*, 403-425.

Suess,W.M. & Berlyne,D.E. (1978). Exploratory behavior as a function of hippocampal damage, stimulus complexity, and stimulus novelty in the hooded rat. *Behavioral Biology, 23*, 487-499.

Surwit,R.S., McCubbin,J.A., Livingston,E.G., & Feinglos,M.N. (1985). Classically conditioned hyperglycemia in the obese mouse. *Psychosomatic Medicine, 47*, 565-568.

Vazdarjanova,A., Cahill,L., & McGaugh,J.L. (2001). Disrupting basolateral amygdala function impairs unconditioned freezing and avoidance in rats. *European Journal of Neuroscience, 14*, 709-718.

Vazdarjanova,A. & McGaugh,J.L. (1998). Basolateral amygdala is not critical for cognitive memory of contextual fear conditioning. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 15003-15007.

Wadenberg,M.-L.G. & Hicks,P.B. (1999). The conditioned avoidance response test re-evaluated: is ti a sensitive test for the detection of potentially atypical antipsychotics? *Neuroscience and Biobehavioral Reviews, 23*, 851-862.

White,N.M. (1998). Cognitive enhancement: an everyday event? *International Journal of Psychology, 33*, 95-105.

White,N.M. & Legree,P. (1984). Effects of posttraining exposure to an aversive stimulus on retention. *Physiological Psychology, 12*, 233-236.

White,N.M. & McDonald,R.J. (2002). Multiple parallel memory systems in the brain of the rat. *Neurobiology of Learning and Memory, 77*, 125-184.

White,N.M. and Ouellet,M.-C. (1997). Roles of movement and temporal factors in spatial learning. *Hippocampus, 7*, 501-510.

Wilensky,A.E., Schafe,G.E., & LeDoux,J.E. (1999). Functional inactivation of the amygdala before but not after auditory fear conditioning prevents memory formation. *The Journal of Neuroscience, 19*, 1-5.

Wilensky,A.E., Schafe,G.E., & LeDoux,J.E. (2000). The amygdala modulates memory consolidation of fear-motivated inhibitory avoidance learning but not classical fear conditioning. *The Journal of Neuroscience, 20*, 7059-7066.

Figure Legends

Figure 1. Mean times (sec ± SEM) spent in the food-paired and unpaired arm locations during CCP testing 24 hours after training (A) or posttraining treatment (B). (A) Groups given 1 (1TT), 2 (2 TT), or 3 (3 TT) training trials on the maze. (B) Groups given 2 training trials on the maze. 15NSh: not shocked during aversive conditioning, exposed to conditioned cues 15 min after CCP training; 15Sh, 30Sh, 2HrSh: shocked during aversive conditioning, exposed to conditioned cues 15 min, 30 min or 2 hr after CCP training, respectively. ** $p < 0.01$, *** $p < 0.001$; food-paired vs food-unpaired times.

Figure 2. (A) Avoidance ratios for rats in 4 training groups (see Figure 1 for abbreviations). The ratios (means ± SEM) are the differences divided by the sums of the times spent in the shock-paired and unpaired compartments. The time required for each rat to leave the paired compartment for the first time was subtracted from each rat's total time in that compartment. (B) Crossover rates are the number of times a rat moved between the two compartments minus 1 divided by the total session time minus the time of the first bout in the paired compartment. ** $p < 0.01$ vs. 15NSh.

Figure 3. Scatterplots showing relationships between the CCP (time in paired arm - time in unpaired arm) and freezing during first paired bout (A), the avoidance ratio (B), and the crossover rate (C). Data points are from individual subjects in groups 15Sh and 30Sh.

Figure 4. Brain sections showing location of injector tips aimed at the amygdala. Implants were located above the central amygdala region. The cannulas did not produce significant damage in any amygdala subregion.

Figure 5. Mean time (sec ± SEM) spent in the food-paired and unpaired arm locations during conditioned preference testing 24 hours after posttraining injections. (A) Groups Given 2 training trials on the maze. Sh-Sal, Sh-Mus: rats were shocked during aversive conditioning and injected with saline or muscimol after CCP training but before exposure to the conditioned aversive cues; NSh-Mus: rats not shocked during aversive conditioning and

injected with muscimol after CCP training but before exposure to the conditioned aversive cues. (B) Groups of rats given 4 CCP training trials and injected with saline or muscimol 15 min later. * $p < 0.05$, *** $p < 0.001$; food-paired vs food-unpaired time.

Figure 6. (A) Avoidance ratio. (B) Crossover rate. Rats injected with saline or muscimol prior to measurement of these responses during exposure to the conditioned stimuli. Data are means ± SEM. Abbreviations as in Figure 5. ** $p < 0.01$ vs. Sh-sal group.

Table 1. Percent Freezing

Experiment 1	Shock-training	Test
15Sh	50.0 ± 3.9	10.4 ± 6.2*, ##
15NSh	2.9 ± 1.4	0.0 ± 0.0
30Sh	50.2 ± 3.8	17.6 ± 7.6*, ##
2HrSh	50.8 ± 3.9	21.9 ± 11.7*, ##
Closed door test		
Shocked	51.4 ± 4.4	73.8 ± 8.0
Non-shocked	11.5 ± 3.8	2.2 ± 1.0
Experiment 2	**Shock-training**	**Test**
Shock-Saline	54.6 ± 6.2	23.2 ± 9.4**, ##
Shock-Muscimol	61.3 ± 6.8	0.0 ± 0.0
NoShock-Muscimol	12.4 ± 2.9	0.0 ± 0.0

Freezing was calculated as the percent of total time on the shock-training day and during the first paired bout of the posttraining exposure to the conditioned cues. Closed door data from Holahan & White, 1999; rats were trained using the identical apparatus and procedure as in the present experiments, but tested while confined in the paired compartment (with the door to the unpaired compartment closed). Experiment 1 abbreviations and in Figure 1. *, $p < 0.05$ vs 15NSh on test day; ** $p < 0.01$ vs Sh-mus and NSh-mus on test day; ## $p < 0.01$ vs shock-training.

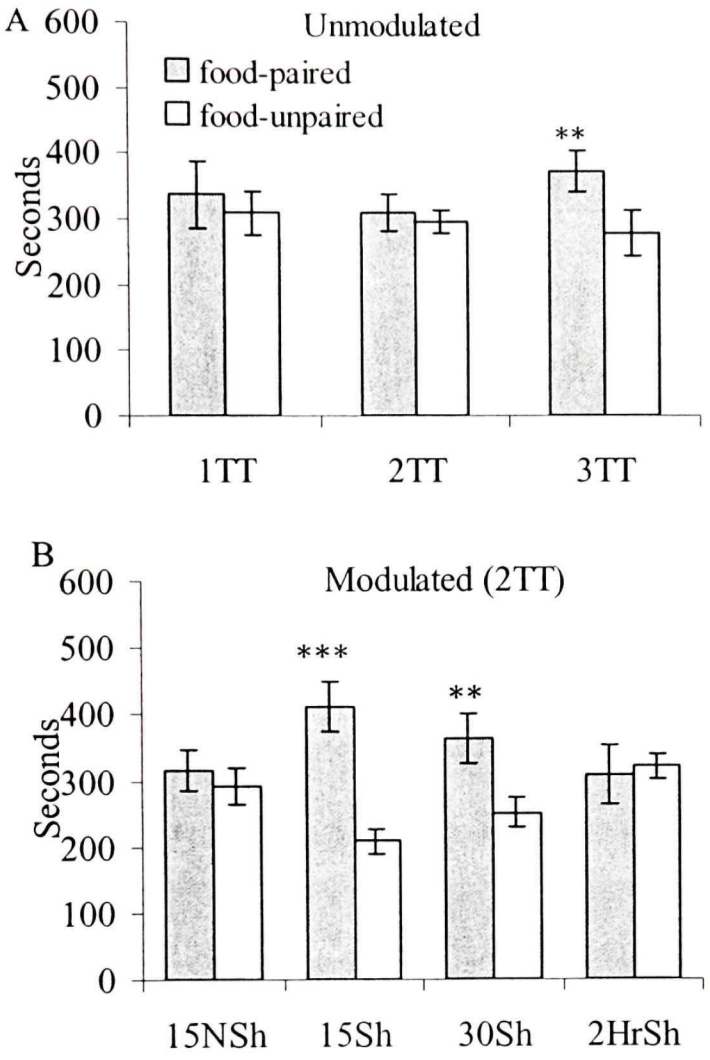

Figure 1 Holahan and White

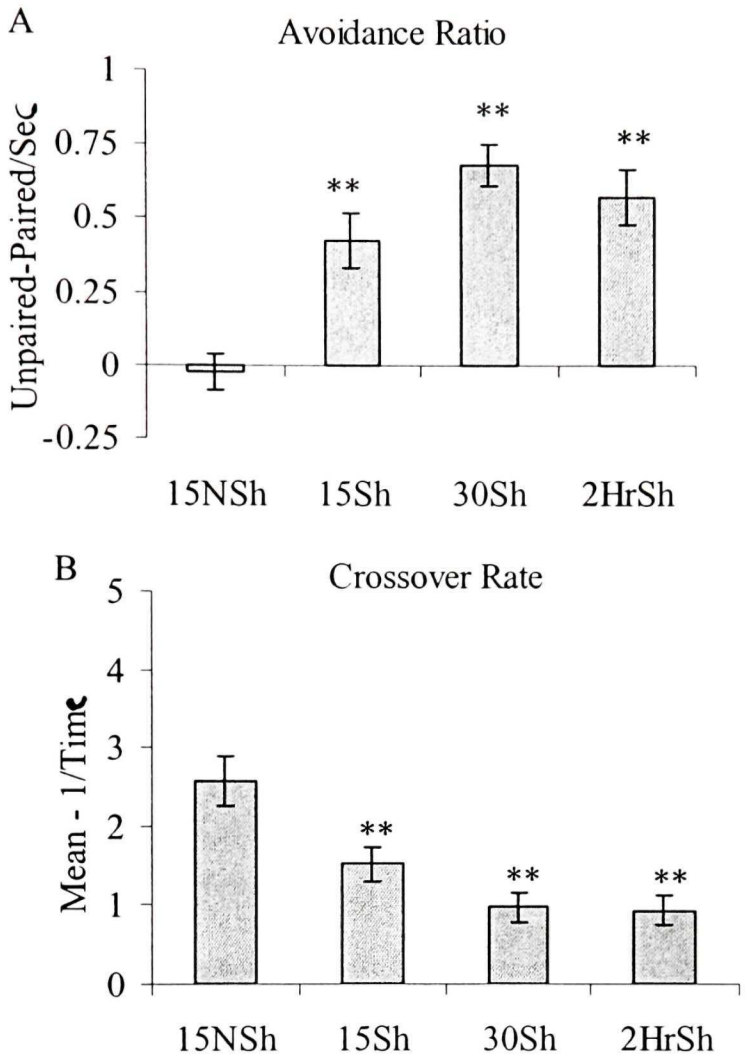

Figure 2 Holahan and White

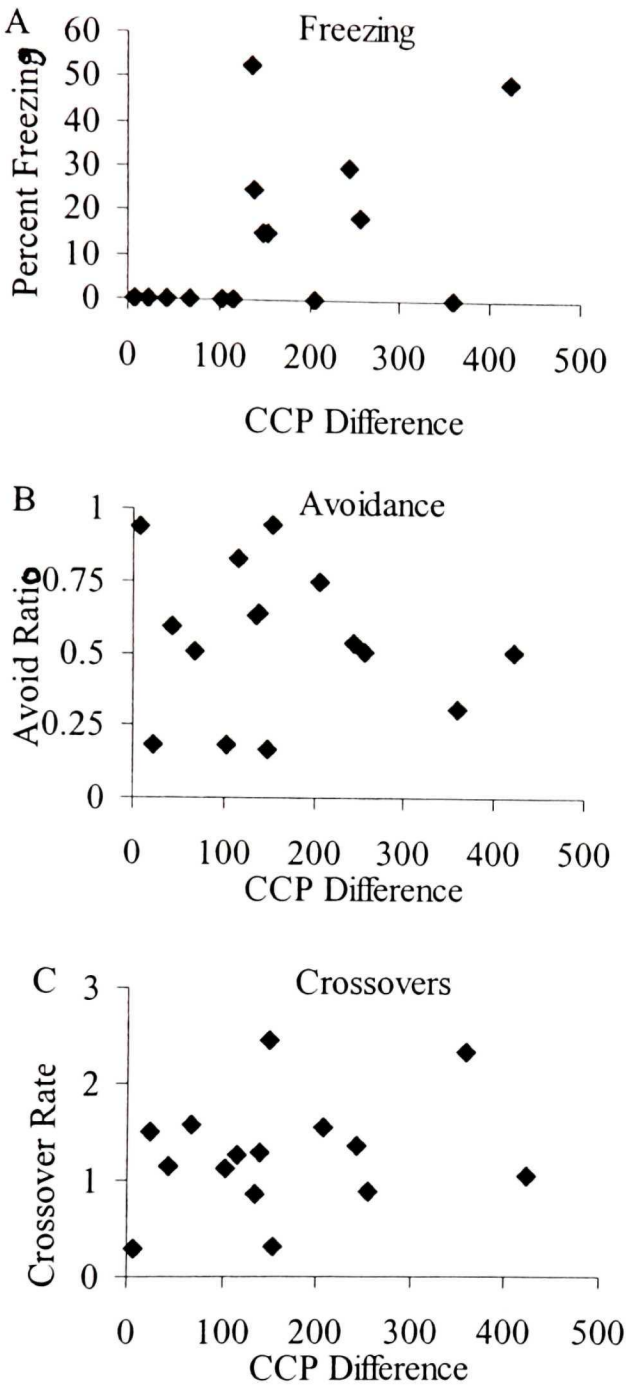

Figure 3 Holahan and White

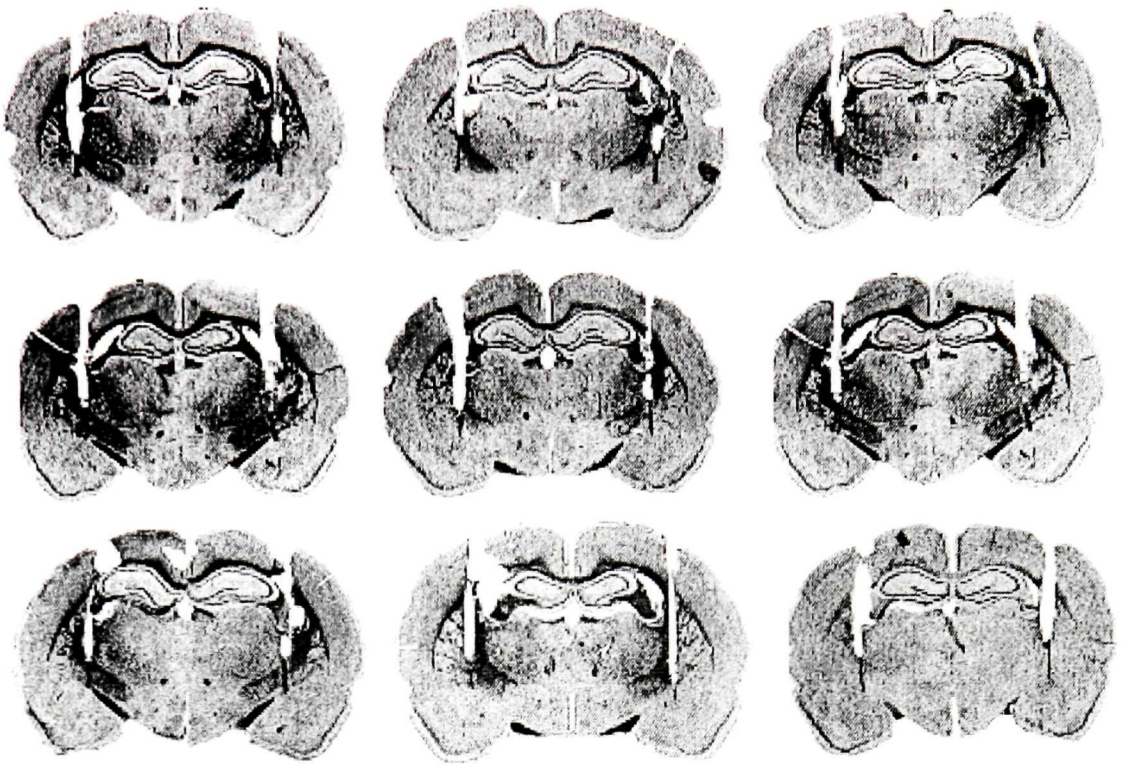

Figure 4 Holahan and White

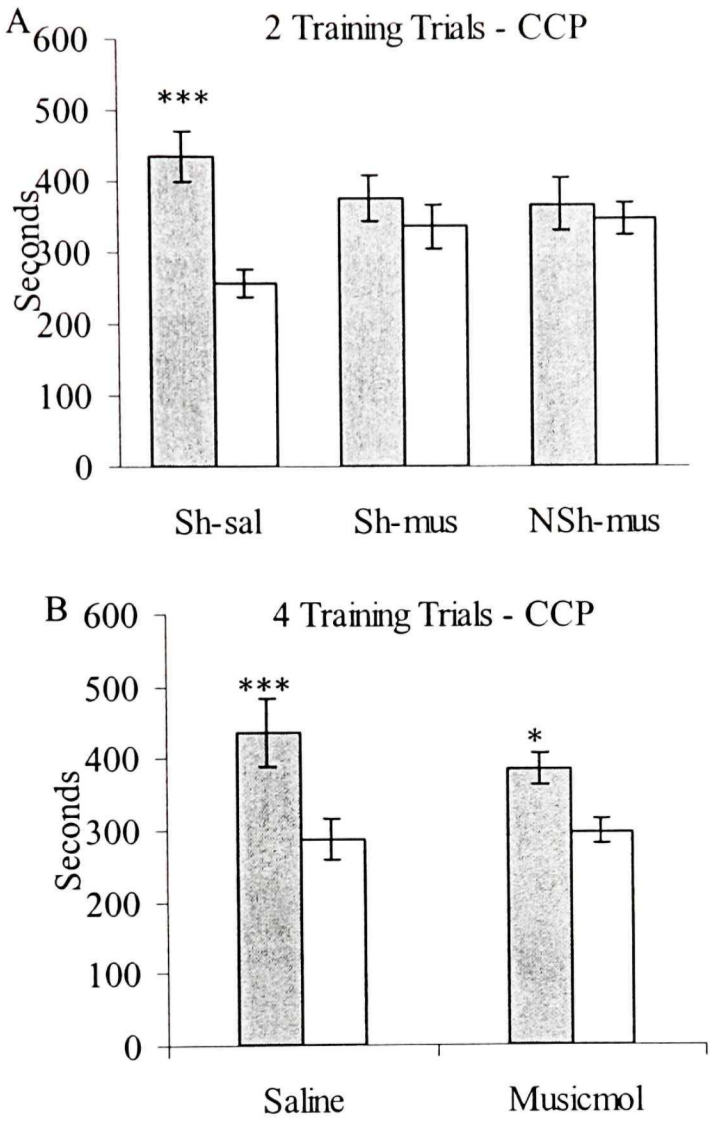

Figure 5 Holahan and White

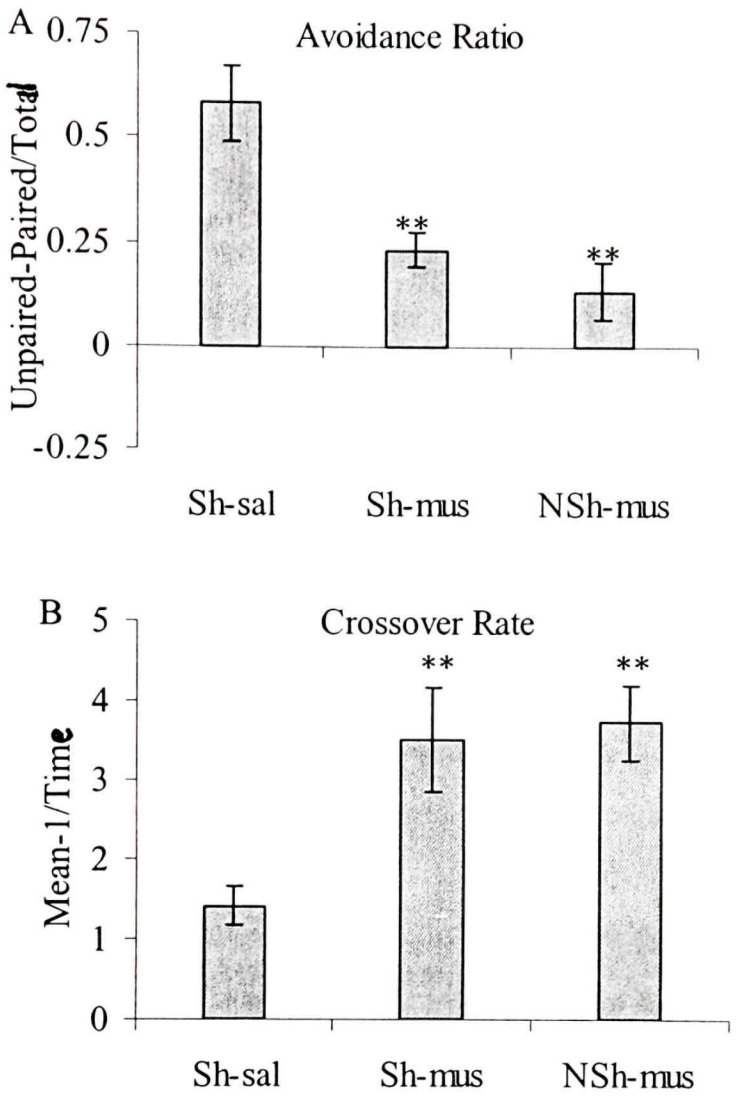

Figure 6 Holahan and White

Discussion

VI. Summary of Results

A. Manuscript 1

Amygdala c-Fos expression was measured following shock (US). Immediately after shock the rats were moved to a neutral compartment (switch condition), which reduced freezing, or left in the shock-paired compartment (stay condition), which elevated freezing. Independently of the stay or switch condition, c-Fos expression was elevated in the central (CeA) and lateral (LA) amygdalae, but not in the basolateral (BLA) or medial (MeA) amygdalae. Since c-Fos was elevated in both conditions, but freezing was elevated only in the shock-stay condition, two conclusions were drawn. First, c-Fos was elevated by shock rather than the post-shock contextual cues. Second, the activation of neurons expressing c-Fos was not based on feedback from freezing nor was it sufficient to produce freezing. It was concluded that the US, but not feedback from freezing, activated c-Fos expressing neurons in the amygdala. Furthermore, this activity does not directly produce freezing.

In the second experiment, rats were shocked and re-exposed either to shock-paired (CS) contextual cues or to unpaired contextual cues. Re-exposure to the contextual CS elevated freezing and BLA, CeA, and LA c-Fos expression compared to the unpaired and no shock groups. Re-exposure to the unpaired contextual cues elevated freezing compared to the no shock group but these two groups had similar levels of c-Fos expression; significantly lower than the group exposed to the CS. This suggested two additional conclusions. Activation of amygdala neurons expressing c-Fos resulted from specific exposure to the CS and not some other stimuli or features of the procedure. Second, freezing can occur in the absence of activation of amygdala neurons expressing c-Fos. The elevated freezing observed when neurons expressing c-Fos were activated suggests the possibility that the activity promoted freezing rather than produced it. Freezing could be produced by other brain regions.

B. Manuscript 2

All rats were shocked in a compartment with the door closed and tested without shock for freezing in the closed door configuration and place avoidance in the open door configuration. Intra-amygdala muscimol injections were given before shock-training or testing to examine the relationship between amygdala inactivation and production of overt

behaviors.

Intra-amygdala muscimol injections suppressed c-Fos expression in the LA/BLA, CeA, and MeA. This indicated that the dose of muscimol was sufficient to suppress neural activity of a population of amygdala neurons expressing c-Fos but precludes any conclusions limited to a specific amygdala subregion. The muscimol injections also spread to the ventral cortex adjacent to the amygdala precluding conclusions limited to the amygdala. Previous results with lesions (Holahan and White, 2002) or muscimol injections (Holahan and White, 2003) restricted to the central amygdala blocked active, place avoidance behavior suggesting that the present results can be attributed to an effect of muscimol in the amygdala rather than the surrounding cortex.

The elevated freezing observed during shock in the group injected with saline was eliminated by intra-amygdala muscimol injections given before training. Pretraining musicmol injections also reduced freezing during the test but freezing in this group increased from the shock phase to the test phase and was higher than in the no shock group. Therefore elimination of freezing during US presentation could have been due to an inability to produce freezing (performance deficit), an attenuated modulation of information processed by another brain region, or blockade of a conditioning process resulting in an attenuated set of internal responses that promote freezing.

The elevated freezing observed in the shock-saline group during testing was eliminated by pretesting intra-amygdala muscimol injections. This effect could have been due to an inability to produce freezing (performance deficit) or a blockade of a conditioning process resulting in an attenuation of internal responses that promote freezing.

Active place avoidance was blocked by both pretraining and pretesting intra-amygdala muscimol injections. Since place avoidance is incompatible with freezing, measurement of both behaviors can control for any performance deficits that may result from a muscimol injection. As both behaviors were blocked with pretraining and pretesting intra-amygdala muscimol injections, the results cannot be interpreted in terms of a performance deficit. Rather, activation of the amygdala may promote the expression of overt behaviors.

C. Manuscript 3

Freezing and place avoidance were measured during, and conditioned memory modulation

was measured 24 hours after exposure to shock-paired contextual cues (CS). Freezing was elevated in some, and place avoidance was elevated in all shocked rats. Posttraining exposure to the CS 15 or 30 minutes but not 2 hours after conditioned cue preference (CCP) training enhanced expression of the CCP 24 hours later. This suggests that exposure to an aversive CS produced an internal response that modulated memory, and promoted the expression of freezing and place avoidance.

Intra-amygdala muscimol injections given before posttraining exposure to the CS blocked the expression of freezing and place avoidance and eliminated conditioned memory modulation. Since posttraining intra-amygdala muscimol injections did not block expression of unmodulated CCP training, amygdala inactivation specifically blocked a conditioned memory modulation process produced by the aversive CS. This suggests that amygdala inactivation blocked an internal conditioned memory modulation response. Since freezing and place avoidance were blocked by the same treatment, it can be suggested that the promotion of these behaviors, when the amygdala is activated by conditioned stimuli, is also due to conditioned internal responses.

VII. Conclusions

The central question addressed in this thesis is whether the amygdala directly produces overt behaviors such as freezing and place avoidance or whether an array of internal responses it produces promote the expression of these behaviors. A second theme relates the hypotheses that the amygdala modulates memories stored in other brain regions and that the amygdala is part of a system that stores memories during aversive conditioning.

A. Production of Overt Behaviors

In the present thesis, overt behaviors are observable changes in motor patterns that occur during exposure to aversive stimuli. These behaviors may be part of an organism's innate behavioral repertoire (Bolles, 1970; 1976) such as freezing but may also include learned behaviors, such as place avoidance, emitted by the organism to reduce exposure to aversive cues (Miller, 1948; Mowrer, 1947; Mowrer and Lamoreaux, 1946). In most cases, these overt behaviors are observed during exposure to an aversive CS.

One hypothesis of amygdala function suggests that it is required for the direct production of specific overt behaviors during aversive conditioning (Cahill, Weinberger, Roozendaal, and

McGaugh, 1999). According to this idea, lesions or inactivations of the amygdala do not interfere with the conditioning process but rather with the ability to perform behaviors that are used as evidence of conditioning.

Amygdala c-Fos expression was not always elevated when the rats froze - an overt behavior (Manuscript 1). If these neurons produced freezing directly, high levels of freezing and c-Fos expression would have coincided. Since this did not occur in the shock-switch or unpaired conditions, activation of CeA and LA neurons expressing c-Fos does not appear to be directly required for the production of freezing. Freezing may require activation of additional neurons expressing other proteins or genes or activation of neurons located in other brain regions.

Activation of amygdala neurons expressing c-Fos does not appear to be sufficient to produce high levels of freezing (shock-switch), and some freezing can occur without activation of amygdala neurons expressing c-Fos (unpaired condition). One possibility is that freezing requires activation of neurons in other brain regions. Lesions of the central gray (Carrive, Lee, and Su, 2000; Carrive, Leung, Harris, and Paxinos, 1997; Carrive, 1993; LeDoux, Iwata, Cicchetti, and Reiss, 1988), the hippocampus (Gewirtz, McNish, and Davis, 2000; McNish, Gewirtz, and Davis, 1997), or the dorsal striatum (Viaud and White, 1989) reduce freezing. Lesions of the central gray (Amorapanth, Nader, and LeDoux, 1999; Carrive, 1993; LeDoux, Iwata, Cicchetti, and Reiss, 1988) or hippocampus (Holahan and White, 2001; McNish, Gewirtz, and Davis, 1997) have been found to specifically block freezing to the exclusion of other overt behaviors such as conditioned suppression (Amorapanth, Nader, and LeDoux, 1999), fear-potentiated startle (McNish, Gewirtz, and Davis, 1997), and place avoidance (Holahan and White, 2001). This suggests that the central gray or the hippocampus may directly produce freezing under certain circumstances.

A second possibility is that there was a population of amygdala neurons expressing another protein whose activation coincided directly with the production of freezing. The c-Fos protein represents a small fraction of inducible gene products (Clayton, 2000; Davis, Bozon, and LaRoche, 2003; Sheng and Greenberg, 1990). It is entirely possible that a different population of amygdala neurons expressing one of these other inducible gene products is required for the direct production of freezing. With this limitation, it is

hypothesized that the direct production of freezing does not require the activation of amygdala neurons that express the c-Fos protein.

Amygdala inactivation before training or testing blocked freezing in the closed door configuration and place avoidance in the open door configuration (Manuscript 2). Previous reports (Helmstetter and Bellgowan, 1994; Muller, Corodimas, Fridel, and LeDoux, 1997) found that pretraining or pretesting intra-amygdala muscimol injections blocked freezing in the presence of an aversive CS. This could have been due to an inability to produce freezing reflecting a performance deficit (Cahill, McGaugh, and Weinberger, 2001; Cahill, Vazdarjanova, and Setlow, 2000; Cahill, Weinberger, Roozendaal, and McGaugh, 1999) or a blockade of a conditioning process (Fanselow and LeDoux, 1999; LeDoux, 2000; Maren, 1999; 2001a). Since intra-amygdala muscimol injections have been found to increase activity levels (pretesting condition in manuscript 2 and Holahan and White, 2003), it is difficult to conclude that the amygdala is specifically involved in a conditioning process when freezing is the only behavior measured.

In the present thesis, performance deficits were controlled for by measuring place avoidance, a behavior that is incompatible with freezing (Anisman, 1973; Anisman and Waller, 1972; 1973). Since place avoidance requires a different behavioral topography than freezing, a deficit in the ability to produce freezing may have left production of place avoidance intact. As both behaviors were attenuated by intra-amygdala muscimol injections, the amygdala does not appear to subserve a direct role in the production of freezing.

A number of overt behaviors are attenuated by lesions of the amygdala. These include freezing (Amorapanth, LeDoux, and Nader, 2000; Holahan and White, 2002; Kim, Rison, and Fanselow, 1993; Maren, 1998; 1999; Nader, Majidishad, Amorapanth, and LeDoux, 2001), passive avoidance (Bermúdez-Rattoni, Introini-Collison, Coleman-Mesches, and McGaugh, 1997; Dunn and Everitt, 1988; Harris and Westbrook, 1995; Parent, Avila, and McGaugh, 1995; Parent, Quirarte, Cahill, and McGaugh, 1995; Parent, Tomaz, and McGaugh, 1992; Parent, West, and McGaugh, 1994), active forms of avoidance (Amorapanth, LeDoux, and Nader, 2000; Killcross, Robbins, and Everitt, 1997; Smith, Monteverde, Schwartz, Freeman, and Gabriel, 2001) including place avoidance (Ambrogi Lorenzini, Bucherelli, Giachetti, Mugnai, and Tassoni, 1991; Antoniadis and McDonald, 2000; Holahan and White, 2002;

Jellestad and Cabrera, 1986), conditioned suppression (Killcross, Robbins, and Everitt, 1997; LeDoux, Iwata, Cicchetti, and Reiss, 1988), and fear-potentiated startle (Campeau and Davis, 1995; Kim, Rison, and Fanselow, 1993). Many of these behaviors (e.g., freezing, passive avoidance, and conditioned suppression) are based on an ability to inhibit ongoing behaviors. In these cases, an amygdala lesion might impair the ability to inhibit behavioral output (i.e., a performance deficit) and result in the observed reduction. On the other hand, active forms of avoidance including place avoidance are based on the ability of an organism to initiate behaviors. In this case, an inability to activate or initiate behavior would produce the observed reduction. Since disrupting amygdala activity blocks both active and passive forms of overt behaviors, these effects are inconsistent with the hypothesis that amygdala inactivations result in specific performance deficits. Therefore, the amygdala does not directly produce overt behaviors used to infer internal responses.

B. Production of Internal Responses

1. From internal responses to overt behaviors

Although they were not directly measured in the present thesis, aversive environmental events are known to elicit a number of internal responses. Foot shock elevates blood glucose (Gold, 1995; Gold, Vogt, and Hall, 1986; Hall and Gold, 1986), norepinephrine release (Gold and McCarty, 1981; Hall and Gold, 1986; Williams, Men, Clayton, and Gold, 1998) and activates the hypothalamic-pituitary-adrenal (HPA) axis (Johnson et al., 2002; Nguyen et al., 1998). Cues previously paired with shock also elevate norepinephrine release (Korte et al., 1992; Korte, Bouws, Koolhaas, and Bohus, 1992) and activate the HPA-axis (Perez and Lysle, 1995; Shurin, Kusnecov, Riechman, and Rabin, 1995). Taken together, these internal responses may constitute the aversive affective state postulated by Cannon (1927), Davis (1997), Freud (1936), Hebb (1946), James (1884), Lazarus (1991), LeDoux (1995), Miller (1948), and Mowrer and Lamoreaux (1946). This state is thought to promote the expression of observable behaviors.

Unconditioned posttraining elevation of norepinephrine (Cahill and McGaugh, 1991; Gold and van Buskirk, 1975), glucose (Gold, Vogt, and Hall, 1986; Messier and White, 1984; 1987; White and Messier, 1988) or activation of the HPA axis (Roozendaal and McGaugh, 1996) modulates memories. It is possible that conditioned elevations in these internal

responses by an aversive CS modulates memory in the same was as unconditioned elevation. Thus, conditioned memory modulation can be seen as resulting from one or more internal responses elicited by aversive CSs. These responses may be among those that constitute an aversive affective state. Therefore, conditioned memory modulation may be a way to infer the existence of conditioned internal responses produced by conditioned aversive stimuli.

It has been suggested (Fanselow and Gale, 2003; Fanselow and LeDoux, 1999; LeDoux, 2000; Maren, 2003; Walker, Toufexis, and Davis, 2003) that the amygdala mediates an affective state which promotes observable behaviors. In the present context, the amygdala is thought to produce the internal responses inferred from memory modulation and the observation of overt behaviors. Consistent with this idea, freezing, place avoidance and conditioned memory modulation produced by posttraining exposure to an aversive CS were blocked by intra-amygdala muscimol injections. This provides a mechanism by which the amygdala can enhance memory consolidation and promote the expression of overt behaviors (see section 2 below).

Further study of the neural and neurochemical bases of conditioned memory modulation may provide a better understanding of amygdala function in aversive conditioning and of the nature and properties of the hypothesized aversive affective state. An examination of the internal responses resulting from aversive and appetitive USs and/or CSs might be used to identify responses that are specific to appetitive or aversive cues and which also contribute to the modulation effect. Some memory modulating consequences of appetitive and aversive USs are the same (increases in blood glucose; Gold, Vogt, and Hall, 1986; Hall and Gold, 1986; Messier and White, 1987; Steffens, 1969; 1970; White and Messier, 1988). Thus conditioned elevations in blood glucose may contribute to the memory modulation effect rather than promote specific internal responses resulting from conditioned aversive or appetitive cues. Other internal responses may be unique to the specific aversive or appetitive properties of the US and/or CS. Similarly, various aversive USs (e.g., shock or cat) may also produce somewhat different internal responses. Clarifying these responses may provide a better understanding of psychological concepts such as "hope" and "fear" and the various uses of these terms (e.g., anxiety, phobia).

2. *From overt behaviors to internal responses*

Since internal responses require specialized procedures to measure, the present thesis and previous authors infer the existence of internal responses from overt behaviors (Brown and Jacobs, 1949; Brown, Kalish, and Farber, 1951; Davis, 1997; Fanselow, 1984; Lazarus, 1991; LeDoux, 2000; Maren, 2001a; Miller, 1948; Mowrer, 1947; Mowrer and Lamoreaux, 1946). In the case of place avoidance, this inference is partly due to the fact that, as in the present experiments, the rats were never trained to perform certain behaviors that occur in aversive conditioning. Training consisted solely of pairing contextual cues with shock; the rats initiated the place avoidance behavior for the first time when they were tested in an apparatus that offered the possibility of avoiding the CS. "Two-factor theory" (McAllister and McAllister, 1995; Miller, 1948; Mowrer, 1947; Rescorla and Solomon, 1967) was originally proposed to explain this phenomenon. According to this theory, when a rat is exposed to an aversive CS, the cues elicit an array of internal responses. It is hypothesized that these internal responses are elicited by cues paired with the aversive event regardless of how they are presented (e.g., the contextual cues in the present experiments were presented in a closed or open door configuration) (Miller, 1948). When internal responses are elicited, overt behaviors occur as an indirect result (Miller, 1948; Mowrer, 1947).

When the door between the two compartments was closed, freezing was elevated and place avoidance could not occur. When the door was open, freezing decreased and place avoidance became the dominant behavior. This suggests that some overt behaviors are sensitive to the environmental configuration. Conditioned memory modulation occurred following posttraining exposure to an aversive CS with the door closed (Holahan and White, 2002) and posttraining exposure to an aversive CS with the door open (Manuscript 3). Posttraining exposure to an aversive CS in the closed door configuration has also been found to modulate the CCP task used in the present thesis (unpublished observations, Holahan and White). This suggests that conditioned memory modulation is not sensitive to the environmental configuration in which the aversive cues are presented. Therefore, depending on the environmental configuration, different behaviors may be observed and each of these behaviors may depend on a different internal response. If this is the case, all behaviors and their corresponding internal responses must be examined to obtain a better understanding of the relationship between overt behaviors and affective states such as fear.

Another aspect of inferring internal responses from overt behaviors is the reliance of overt behaviors on performance variables. Injections of amphetamine, which increase activity (Kelly, 1977), are reported to reduce the occurrence of freezing (Bindra and Anchel, 1963). This suggests that the ability to freeze is sensitive to a competing behavior. When a competing behavior, such as increased activity, interferes with the ability to perform an overt behavior, such as freezing, alterations in the observed behavior may not accurately reflect the internal state that promotes it. It has also been found that elevations in freezing interfere with the ability of rats to initiate active avoidance (Manuscripts 2 and 3; Anisman, 1973; Anisman and Waller, 1973; Holahan and White, 2001; Sidman, 1962a; 1962b). If these forms of interference reduce the ability to perform an overt behavior, the relationship between overt behaviors and internal responses that produce them may not be linear, making inferences inaccurate.

Posttraining administration of a number of substances modulate memory (for reviews see Cahill and McGaugh, 1996; McGaugh and Cahill, 1997; McGaugh, Cahill, Ferry, and Roozendaal, 2000; White, 1998). Amphetamine (Carr and White, 1984; Evangelista and Izquierdo, 1971; Johnson and Waite, 1971), glucose (Gold, Vogt, and Hall, 1986; Messier and White, 1984; 1987) shock (Holahan and White, 2002; Jodar, Takahashi, and Kaneto, 1996; White and Legree, 1984), and shock-paired cues (Holahan and White, 2002; Manuscript 3) all modulate memory. Neither amphetamine nor glucose elicit freezing (Kelly, 1977 and personal observations), shock elicits moderate levels of freezing (Blanchard and Blanchard, 1969b; Bolles and Collier, 1976; Fanselow, 1980; Manuscript 1) and shock-paired cues elicit high levels of freezing (Manuscript 1 and 2). This suggests that an inability to freeze or initiate movement does not influence conditioned memory modulation.

It could be argued that freezing is necessary for memory modulation with aversive CSs. The lack of a correlation between freezing and conditioned memory modulation suggests that freezing is not required to observe conditioned memory modulation. It must be kept in mind that determining the true relationship between the two behavioral measures used here (freezing and the CCP used to detect memory modulation) may be complicated by the low number of subjects. The relationship should be further examined by increasing the number of subjects. Varying the levels of shock during conditioning would also help to clarify the

relationship between the two measures.

The arguments above suggest that conditioned memory modulation probably does not depend on the environmental configuration or the ability to perform specific behaviors. Therefore, blockade of conditioned memory modulation during exposure to the aversive CS with intra-amygdala muscimol injections may have been due to blockade of an array of internal responses. These amygdala-mediated internal responses that modulate memory may also be required for the promotion of other overt behaviors. Interactions with other brain regions such as the brain stem central gray area (Carrive, Lee, and Su, 2000; Carrive, Leung, Harris, and Paxinos, 1997; Carrive, 1993; LeDoux, Iwata, Cicchetti, and Reiss, 1988) or the dorsal striatum (Brasted, Döbrössy, Robbins, and Dunnett, 1998; Brasted, Humby, Dunnett, and Robbins, 1997; Brown and Robbins, 1989; Hauber and Schmidt, 1994; Viaud and White, 1989; White and Salinas, 2003) may be required for the specific inhibition or initiation of overt behaviors.

C. Memory Storage or Memory Modulation

It has been suggested that the amygdala stores memories during aversive conditioning (Fanselow and Gale, 2003; Fanselow and LeDoux, 1999; Maren, 1999; 2003). According to one recent theory (White and McDonald, 2002), memories are stored in neural systems and damage to major components of these systems impairs their mnemonic functions. Evidence from studies of aversive conditioning, including those in the present thesis, is consistent with the idea that the amygdala is a critical part of a system that mediates memories of the Pavlovian type, in which the conditioned response may be an array of internal responses (White and McDonald, 2002).

The observation of c-Fos-expressing neurons following both US and CS presentation (Manuscript 1) suggests that some plasticity-related changes may have occurred within the amygdala. The c-Fos protein regulates the transcription of additional genes that produce structural or functional neuronal changes (Angle and Karin, 1991; Carew, 1996; Goelet, Castellucci, Schacher, and Kandel, 1986; Rose, 1991; 1996; 2000; Sheng and Greenberg, 1990). These changes may reflect synaptic mechanisms underlying behavioral changes associated with learning and memory. The c-Fos protein may be a marker for neurons that have undergone structural or functional changes associated with plasticity or learning-related

processes.

The initial increase in CeA and LA c-Fos expression following shock (Manuscript 1) is hypothesized to reflect structural or functional changes associated with the acquisition of a US representation. Previous reports have found that c-Fos expression in the amygdala is sensitive to other aversive USs (Beckett, Duxon, Aspley, and Marsden, 1997; Campeau, *et al.*, 1991; Milanovic, *et al.*, 1998; Radulovic, Kammermeier, and Spiess, 1998). This suggests that the functionality of a population of amygdala neurons expressing c-Fos may be altered by aversive USs (Stork, Stork, Pape, and Obata, 2001) and hence, store a representation of these USs (Gilbert and Kesner, 2002; Kesner and Gilbert, 2001).

Elevated c-Fos expression in the amygdala following re-exposure to the CS is hypothesized to reflect activation of the US representation. Animals exposed to explicitly paired but not unpaired contextual cues showed elevated amygdala c-Fos expression. It is further suggested that the contextual information is processed by other brain regions such as perirhinal, parahippocampal, or entorhinal cortices (Pitkänen, 2000; Turner, Mishkin, and Knapp, 1980; van Hoesen, 1981) that send inputs to the amygdala. According to this scheme, connections between these regions and the amygdala would represent the association between the CS and US. The representation of the contextual CS would be stored in the cortical association areas and the representation of the US would be stored in synaptic relationships between amygdala neurons.

There are two possible reasons why neuroplastic events would occur during memory retrieval. One is that c-Fos is a marker for additional plasticity-related changes underlying long-term storage (Nader, Schafe, and LeDoux, 2000a; 2000b). The US representation would be activated during exposure to the CS and have to undergo a second consolidation process requiring protein synthesis (Nader, 2003; Nader, Schafe, and LeDoux, 2000a). A second possibility is that the c-Fos protein is a marker for the extinction of the initial US representation (Baker and Azorlosa, 1996; Berman and Dudai, 2001; Falls, Miserendino, and Davis, 1992; Lu, Walker, and Davis, 2001; Walker and Davis, 2002). As argued by others (Baker and Azorlosa, 1996; Berman and Dudai, 2001; Bouton, 1994; Falls, Miserendino, and Davis, 1992; Rescorla, 1996; Vianna, Szapiro, McGaugh, Medina, and Izquierdo, 2001) extinction requires new learning. This new learning may require similar cellular mechanisms

to the initial learning.

Pretraining and pretesting intra-amygdala muscimol injections blocked two incompatible behaviors, freezing and place avoidance. It is possible that the behavioral deficit produced by the pretraining injections was due to a lack of memory modulation produced by the shock. Both the dorsal striatum and the hippocampus are modulated by the amygdala (Packard, Cahill, and McGaugh, 1994; Packard and Teather, 1998). Lesions of the dorsal striatum block freezing (Viaud and White, 1989) and active avoidance (Kirkby and Kimble, 1968; Kirkby and Polgar, 1974). Lesions of the hippocampus block contextual freezing (Antoniadis and McDonald, 2000; Kim and Fanselow, 1992; Kim, Rison, and Fanselow, 1993; McNish, Gewirtz, and Davis, 1997; Phillips and LeDoux, 1992) and active place avoidance (Antoniadis and McDonald, 2000; Isaacson, Douglas, and Moore, 1961; Selden, Everitt, Jarrard, and Robbins, 1991). This suggests that the amygdala may have modulated information processing in the dorsal striatum and hippocampus during conditioning. A behavioral deficit would result from a blockade of amygdala-mediated modulation.

Pretesting intra-amygdala muscimol injections eliminated conditioned memory modulation, freezing, and place avoidance (Manuscript 3). As already discussed, the elimination of these three measures can not be accounted for in terms of a performance deficit. It is also not plausible to account for these deficits solely in terms of modulation. If the amygdala served only to modulate information processed by other brain regions, a pretesting injection should have had no effect. As this was not the case, limiting the function of the amygdala to modulation is not valid. It may also serve to store certain memories during aversive conditioning.

Localizing the storage of memories is a notoriously difficult problem. The evidence described above, taken together with the hypothesis that the amygdala mediates production of internal responses leaves us with the difficulty of dissociating the memory from the conditioned response. It may not be possible to distinguish between the hypotheses that a lesion of the amygdala blocks production of conditioned internal responses and that it eliminates the neural substrate of a memory. Possibly the issue is moot - if a representation of the CR is an integral part of a Pavlovian association, there may be no distinction between eliminating the memory and eliminating the response it produces.

References

Amaral, D. G., Price, J. L., Pitkänen, A., & Carmichael, S. T. (1992). Anatomical organization of the primate amygdaloid complex. In J.P.Aggleton (Ed.), The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction. (pp. 1-66). New York: Wiley-Liss.

Ambrogi Lorenzini, C., Bucherelli, C., Giachetti, A., Mugnai, L., & Tassoni, G. (1991). Effects of nucleus basolateralis amygdalae neurotoxic lesions on aversive conditioning in the rat. *Physiology and Behavior, 49*, 765-770.

Ammassari-Teule, M., Pavone, F., Castellano, C., & McGaugh, J. L. (1991). Amygdala and dorsal hippocampus lesions block the effects of GABAergic drugs on memory storage. *Brain Research, 551*, 104-109.

Amorapanth, P., LeDoux, J. E., & Nader, K. (2000). Different lateral amygdala outputs mediate reactions and actions elicited by a fear-arousing stimulus. *Nature Neuroscience, 3*, 74-79.

Amorapanth, P., Nader, K., & LeDoux, J. E. (1999). Lesions of the periacqueductal gray dissociate-conditioned freezing from conditioned suppression behavior in rats. *Learning and Memory, 6*, 491-499.

Angle, P. & Karin, M. (1991). The role of Jun, Fos and the AP-1 complex in cell proliferation and transformation. *Biochimica et Biophysica Acta, 1072*, 129-157.

Anisman, H. (1973). Effects of pretraining compatible and incompatible responses on subsequent one-way and shuttle-avoidance performance in rats. *Journal of Comparative and Physiological Psychology, 82*, 95-104.

Anisman, H. & Waller, T. G. (1972). Facilitative and disruptive effects of prior exposure to shock on subsequent avoidance performance. *Journal of Comparative and Physiological Psychology, 78*, 113-122.

Anisman, H. & Waller, T. G. (1973). Effects of inescapable shock on subsequent avoidance performance: role of response repertoire changes. *Behavioral Biology, 9*, 331-355.

Antoniadis, E. A. & McDonald, R. J. (1999). Discriminative fear conditioning to context expressed by multiple measures of fear in the rat. *Behavioural Brain Research, 101*, 1-13.

Antoniadis, E. A. & McDonald, R. J. (2000). Amygdala, hippocampus and discriminative fear

conditioning to context. *Behavioural Brain Research, 108*, 1-19.

Baker, J. D. & Azorlosa, J. L. (1996). The NMDA antagonist MK-801 blocks the extinction of Pavlovian fear conditioning. *Behavioral Neuroscience, 110*, 618-620.

Barbas, H. & de Olmos, J. (1990). Projections from the amygdala to basoventral and mediodorsal prefrontal regions in the rhesus monkey. *Journal of Comparative Neurology, 300*, 549-571.

Baron, A. (1964). Suppression of exploratory behavior by aversive stimulation. *Journal of Comparative and Physiological Psychology, 57*, 299-301.

Beck, C. H. M. & Fibiger, H. C. (1995). Conditioned fear-induced changes in behavior and in the expression of the immediate early gene *c-fos*: with and without diazepam pretreatment. *The Journal of Neuroscience, 15*, 709-720.

Beckett, S. R. G., Duxon, M. S., Aspley, S., & Marsden, C. A. (1997). Central c-Fos expression following 20kHz/ultrasound induced defence behaviour in the rat. *Brain Research Bulletin, 42*, 421-426.

Benowitz, L. I. & Routtenberg, A. (1997). GAP-43: an intrinsic determinant of neuronal development and plasticity. *Trends in Neurosciences, 20*, 84-91.

Berman, D. E. & Dudai, Y. (2001). Memory extinction, learning anew, and learning the new: dissociations in the molecular machinery of learning in cortex. *Science, 291*, 2417-2419.

Berman, R. F. & Kesner, R. P. (1976). Posttrial hippocampal, amygdaloid, and lateral hypothalamic electrical stimulation: effects on short- and long-term memory of an appetitive experience. *Journal of Comparative and Physiological Psychology, 90*, 260-267.

Bermúdez-Rattoni, F., Introini-Collison, I., Coleman-Mesches, K., & McGaugh, J. L. (1997). Insular cortex and amygdala lesions induced after aversive training impair retention: effects of degree of training. *Neurobiology of Learning and Memory, 67*, 57-63.

Bindra, D. & Anchel, H. (1963). Immobility as an avoidance response, and its disruption by drugs. *Journal of the Experimental Analysis of Behavior, 6*, 213-218.

Blanchard, D. C. & Blanchard, R. J. (1972). Innate and conditioned reactions to threat in rats with amygdaloid lesions. *Journal of Comparative and Physiological Psychology, 81*, 281-290.

Blanchard, D. C. & Takahashi, S. N. (1988). No change in intermale aggression after amygdala lesions which reduce freezing. *Physiology and Behavior, 42*, 613-616.

Blanchard, R. J. & Blanchard, C. (1968a). Escape and avoidance responses to a fear eliciting situation. *Psychonomic Sciences, 13*, 19-20.

Blanchard, R. J. & Blanchard, C. (1969b). Crouching as an index of fear. *Journal of Comparative and Physiological Psychology, 67*, 370-375.

Blanchard, R. J. & Blanchard, D. C. (1968c). Escape and avoidance responses to a fear eliciting situation. *Psychonomic Sciences, 13*, 19-20.

Blanchard, R. J. & Blanchard, D. C. (1968b). Passive avoidance: a variety of fear conditioning? *Psychonomic Sciences, 13*, 17-18.

Blanchard, R. J. & Blanchard, D. C. (1969a). Passive and active reactions to fear-eliciting stimuli. *Journal of Comparative and Physiological Psychology, 68*, 129-135.

Blanchard, R. J. & Blanchard, D. C. (1970a). Dual mechanisms in passive avoidance: I. *Psychonomic Sciences, 19*, 1-2.

Blanchard, R. J. & Blanchard, D. C. (1970b). Dual mechanisms in passive avoidance: II. *Psychonomic Sciences, 19*, 3-4.

Blanchard, R. J. & Blanchard, D. C. (1971). Defensive reactions in the albino rat. *Learning and Motivation, 2*, 351-362.

Blanchard, R. J., Dielman, T. E., & Blanchard, D. C. (1968). Prolonged aftereffects of a single foot shock. *Psychonomic Sciences, 10*, 327-328.

Blanchard, R. J., Fukunaga, K. K., & Blanchard, D. C. (1976). Environmental control of defensive reactions to footshock. *Bulletin of the Psychonomic Society, 8*, 129-130.

Bolles, R. C. & Collier, A. C. (1976). The effect of predictive cues on freezing in rats. *Animal Learning and Behavior, 4*, 6-8.

Bolles, R. C. (1970). Species-specific defensive reactions and avoidance learning. *Psychological Review, 77*, 32-48.

Bolles, R. C. (1976). Theory of Motivation. (2 ed.) New York: Harper and Row.

Bouton, M. E. (1993). Context, time, and memory retrieval in the interference paradigms of Pavlovian learning. *Psychological Bulletin, 114*, 80-99.

Bouton, M. E. (1994). Conditioning, remembering, and forgetting. *Journal of Experimental*

Psychology: Animal Behavior Processes, 20, 219-231.

Bouton, M. E. & Bolles, R. C. (1980). Conditioned fear assessed by freezing and by the suppression of three different baselines. *Animal Learning and Behavior, 8*, 429-434.

Bovet, D., McGaugh, J. L., & Oliverio, A. (1966). Effects of post trial administration of drugs on avoidance learning of mice. *Life Sciences, 5*, 1309-1315.

Brace, H., Latimer, M., & Winn, P. (1997). Neurotoxicity, blood-brain barrier breakdown, demyelination and remyelination associated with NMDA-induced lesions of the rat lateral hypothalamus. *Brain Research Bulletin, 43*, 447-455.

Brasted, P. J., Döbrössy, M. D., Robbins, T. W., & Dunnett, S. B. (1998). Striatal lesions produce distinctive impairments in reaction time performance in two different operant chambers. *Brain Research Bulletin , 46*, 487-493.

Brasted, P. J., Humby, T., Dunnett, S. B., & Robbins, T. W. (1997). Unilateral lesions of the dorsal striatum in rats disrupt responding in egocentric space. *The Journal of Neuroscience, 17*, 8919-8926.

Breen, R. A. & McGaugh, J. L. (1961). Facilitation of maze learning with posttrial injections of picrotoxin. *Journal of Comparative and Physiological Psychology, 54*, 498-501.

Brener, J. & Goesling, W. J. (1970). Avoidance conditioning of activity and immobility in rats. *Journal of Comparative and Physiological Psychology, 70*, 276-280.

Brown, J. S. & Jacobs, A. (1949). The role of fear in the motivation and acquisition of responses. *The Journal of Experimental Psychology, 39*, 747-759.

Brown, J. S., Kalish, H. I., & Farber, I. E. (1951). Conditioned fear as revealed by magnitude of startle response to an auditory stimulus. *Journal of Experimental Psychology, 41*, 317-328.

Brown, V. J. & Robbins, T. W. (1989). Elementary processes of response selection mediated by distinct regions of the striatum. *The Journal of Neuroscience, 9*, 3760-3765.

Bucherelli, C., Tassoni, G., & Bures, J. (1992). Time-dependent disruption of passive avoidance acquisition by post-training intra-amygdala injections of tetrodotoxin in rats. *Neuroscience Letters, 140*, 231-234.

Burns, L. H., Annett, L., Kelley, A. E., Everitt, B. J., & Robbins, T. W. (1996). Effects of lesions to the amygdala, ventral subiculum, medial prefrontal cortex, and nucleus

accumbens on the reaction to novelty: implications for limbic-striatal interactions. *Behavioral Neuroscience, 110*, 60-73.

Burns, L. H., Robbins, T. W., & Everitt, B. J. (1993). Differential effects of excitotoxic lesions of the basolateral amygdala, ventral subiculum, and medial prefrontal cortex on responding with conditioned reinforcement and locomotor activity potentiated by intra-accumbens infusions of D-amphetamine. *Behavioural Brain Research, 55*, 167-183.

Burstein, R. & Potrebic, S. (1993). Retrograde labeling of neurons in the spinal cord that project directly to the amygdala or the orbital cortex in the rat. *The Journal of Comparative Neurology, 335*, 469-485.

Cahill, L. & McGaugh, J. L. (1991). NMDA-induced lesions of the amygdaloid complex block the retention-enhancing effect of posttraining epinephrine. *Psychobiology, 19*, 206-210.

Cahill, L. & McGaugh, J. L. (1996). Modulation of memory storage. *Current Opinion in Neurobiology, 6*, 237-242.

Cahill, L., McGaugh, J. L., & Weinberger, N. M. (2001). The neurobiology of learning and memory: some reminders to remember. *Trends in Neurosciences, 24*, 578-581.

Cahill, L., Vazdarjanova, A., & Setlow, B. (2000). The basolateral complex is involved with, but is not necessary for, rapid acquisition of Pavlovian 'fear conditioning'. *European Journal of Neuroscience, 12*, 3044-3050.

Cahill, L., Weinberger, N. M., Roozendaal, B., & McGaugh, J. L. (1999). Is the amygdala a locus of "conditioned fear"? Some questions and caveats. *Neuron, 23*, 327-328.

Campbell, B. A. & Campbell, E. H. (1962). Retention and extinction of learned fear in infant and adult rats. *Journal of Comparative and Physiological Psychology, 55*, 1-8.

Campeau, S. & Davis, M. (1995). Involvement of the central nucleus and basolateral complex of the amygdala in fear conditioning measured with fear-potentiated startle in rats trained concurrently with auditory and visual stimuli. *The Journal of Neuroscience, 15*, 2301-2311.

Campeau, S., Hayward, M. D., Hope, B. T., Rosen, J. B., Nestler, E. J., & Davis, M. (1991). Induction of the *c-fos* proto-oncogene in rat amygdala during unconditioned and conditioned fear. *Brain Research, 565*, 349-352.

Campeau, S., Miserendino, M. J. D., & Davis, M. (1992). Intra-amygdala infusion of the *N*-Methyl-D-Aspartate receptor antagonist AP5 blocks acquisition but not expression of fear-potentiated startle to an auditory conditioned stimulus. *Behavioral Neuroscience, 106*, 569-574.

Cannon, W. B. (1927). The James-Lange theory of emotion: a critical examination and an alternative theory. *American Journal of Psychology, 39*, 106-124.

Canteras, N. S., Chiavegatto, S., Ribeiro do Valle, L. E., & Swanson, L. W. (1997). Severe reduction of rat defensive behavior to a predator by discrete hypothalamic chemical lesions. *Brain Research Bulletin, 44*, 297-305.

Carew, T. J. (1996). Molecular memory enhancement of memory formation. *Neuron, 16*, 5-8.

Carr, G. D. & White, N. M. (1984). The relationship between stereotypy and memory improvement produced by amphetamine. *Psychopharmacology, 82*, 203-209.

Carrive, P., Lee, J., & Su, A. (2000). Lidocaine blockade of amygdala output in fear-conditioned rats reduces Fos expression in the ventrolateral periaqueductal gray. *Neuroscience, 95*, 1071-1080.

Carrive, P., Leung, P., Harris, J., & Paxinos, G. (1997). Conditioned fear to context is associated with increased Fos expression in the caudal ventrolateral region of the midbrain periacqueductal gray. *Neuroscience, 78*, 165-177.

Carrive, P. (1993). The periacqueductal gray and defensive behavior: functional representation and neuronal organization. *Behavioural Brain Research, 58*, 27-47.

Chacto, C. & Lubow, R. E. (1967). Classical conditioning and latent inhibition in the white rat. *Psychonomic Sciences, 9*, 135-136.

Christophersen, E. R. & Denny, M. R. (1967). Retractable-bar avoidance. *Psychonomic Sciences, 9*, 579-580.

Cimadevilla, J. M., Fenton, A. A., & Bures, J. (2000b). Continuous place avoidance task reveals differences in spatial navigation in male and female rats. *Behavioural Brain Research, 107*, 161-169.

Cimadevilla, J. M., Fenton, A. A., & Bures, J. (2000a). Functional inactivation of dorsal hippocampus impairs active place avoidance in rats. *Neuroscience Letters, 285*, 53-56.

Clayton, D. F. (2000). The genomic action potential. *Neurobiology of Learning and Memory*,

74, 185-216.

Coar, T. (1982). The aphorisms of Hippocrates: with a translation into Latin and English by Thomas Coar. Birmingham, Alabama: Classics of Medicine Library.

Cousens, G. & Otto, T. (1998). Both pre- and posttraining excitotoxic lesions of the basolateral amygdala abolish the expression of olfactory and contextual fear conditioning. *Behavioral Neuroscience*, *112*, 1092-1103.

Darwin, C. (1872). The expression of emotions in man and animals. London, UK.

Davis, M. (1992). The role of the amygdala in conditioned fear. In J.P.Aggleton (Ed.), The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction. (pp. 255-305). New York: Wiley-Liss.

Davis, M. (1997). Neurobiology of fear responses: the role of the amygdala. *Journal of Neuropsychiatry and Clinical Neurosciences*, *9*, 382-402.

Davis, M. (2000). The role of the amygdala in conditioned and unconditioned fear and anxiety. In J.P.Aggleton (Ed.), The Amygdala: Second Edition: A Functional Analysis (2 ed., pp. 213-287). Oxford: Oxford University Press.

Davis, S., Bozon, B., & LaRoche, S. (2003). How necessary is the activation of the immediate early gene *zif 268* in synaptic plasticity and learning. *Behavioural Brain Research*, *142*, 17-30.

de Olmos, J., Alheid, G. F., & Beltramino, C. A. (1985). Amygdala. In The Rat Nervous System (pp. 223-334). Sidney: Academic Press.

Dunn, L. T. & Everitt, B. J. (1988). Double dissociation of the effects of amygdala and insular cortex lesions on conditioned tast aversion, passive avoidance, and neophobia in the rat using the excitotoxin ibotenic acid. *Behavioral Neuroscience*, *102*, 3-23.

Evangelista, A. M. & Izquierdo, I. (1971). The effect of pre- and post-trial amphetamine injections on avoidance responses in rats. *Psychopharmacologia*, *20*, 42-47.

Falls, W. A., Miserendino, M. J. D., & Davis, M. (1992). Extinction of fear-potentiated startle: blockade by infusion of an NMDA antagonist into the amygdala. *The Journal of Neuroscience*, *12*, 854-863.

Fanselow, M. S. (1980). Conditional and unconditional components of postshock freezing. *Pavlovian Journal of Biological Sciences*, *15*, 177-182.

Fanselow, M. S. (1982). The postshock activity burst. *Animal Learning and Behavior, 10,* 448-454.

Fanselow, M. S. (1984). What is conditioned fear? *Trends in Neurosciences, 7,* 460-462.

Fanselow, M. S. (1986). Associative vs topographical accounts of the immediate shock-freezing deficit in rats: implications for the response selection rules governing species-specific defensive reactions. *Learning and Motivation, 17,* 16-39.

Fanselow, M. S. (2000). Contextual fear, gestalt memories, and the hippocampus. *Behavioural Brain Research, 110,* 73-81.

Fanselow, M. S. & Gale, G. D. (2003). The amygdala, fear, and memory. *Annals of the New York Academy of Sciences, 985* , 125-134.

Fanselow, M. S. & Kim, J. J. (1994). Acquisition of contextual Pavlovian fear conditioning is blocked by application of an NMDA receptor antagonist D,L-2-Amino-5-Phosphonovaleric Acid to the basolateral amygdala. *Behavioral Neuroscience, 108,* 210-212.

Fanselow, M. S., Landeira-Fernandez, J., DeCola, J. P., & Kim, J. J. (1994). The immediate-shock deficit and postshock analgesia: implications for the relationship between the analgesic CR and UR. *Animal Learning and Behavior, 22,* 72-76.

Fanselow, M. S. & LeDoux, J. E. (1999). Why we think plasticity underlying Pavlovian fear conditioning occurs in the basolateral amygdala. *Neuron, 23,* 229-232.

Fendt, M. (2001). Injections of the NMDA receptor antagonist aminophosphonopentanoic acid into the lateral nucleus of the amygdala block the expression of fear-potentiated startle and freezing. *The Journal of Neuroscience, 21,* 4111-4115.

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

File, S. E., Zangrossi, H., Sanders, F. L., & Mabbutt, P. S. (1993). Dissociation between behavioral and corticosterone responses on repeated exposures to cat odor. *Physiology and Behavior, 54,* 1109-1111.

Flint, R. W., Metzger, M. M., Benson, D. M., & Riccio, D. C. (1997). Stress-induced memory enhancement for inhibitory fear conditioning in rats. *Psychobiology, 25,* 89-94.

Fortenbaugh, W. W. (2002). Aristotle on emotion: a contribution to philosophical

psychology, rhetoric, poetics, politics, and ethics. (2 ed.) London, UK: Duckworth.

Freud, S. (1936). The problem of anxiety. New York: Norton and Co.

Friedman, D. P., Murray, E. A., O'Neil, J. B., & Mishkin, M. (1986). Cortical connections of the somatosensory fields on the lateral sulcus of macaques: evidence for a corticolimbic pathway for touch. *Journal of Comparative Neurology, 252*, 323-347.

Gallagher, M. & Kapp, B. S. (1978). Manipulation of the opiate activity in the amygdala alters memory processes. *Life Sciences, 23*, 1973-1978.

Gallagher, M., Kapp, B. S., Musty, R. E., & Driscoll, P. A. (1977). Memory formation: evidence for a specific neurochemical system in the amygdala. *Science, 198*, 423-425.

Gaston, M. G. & Freed, L. (1969). Effect of amygdaloid lesions in a fear conditioning situation not involving instrumental learning. *Psychonomic Sciences, 16*, 55-56.

Gerard, R. W. (1961). The fixation of experience. In A.Fessard, R. W. Gerard, & J. Konorski (Eds.), Brain Mechanisms and Learning: A Symposium (pp. 21-35). Oxford: Blackwell Scientific Publications.

Gewirtz, J. C., McNish, K. A., & Davis, M. (2000). Is the hippocampus necessary for contextual fear conditioning? *Behavioural Brain Research, 110*, 83-95.

Gilbert, P. E. & Kesner, R. P. (2002). The amygdala but not the hippocampus is involved in pattern separation based on reward value. *Neurobiology of Learning and Memory, 77*, 338-353.

Glickman, S. E. (1961). Perservative neural processes and consolidation of the memory trace. *Psychological Bulletin, 58*, 218-233.

Goddard, G. V. (1964). Amygdaloid stimulation and learning in the rat. *Journal of Comparative and Physiological Psychology, 52*, 23-30.

Goelet, P., Castellucci, V. F., Schacher, S., & Kandel, E. R. (1986). The long and the short of long-term memory - a molecular framework. *Nature, 322*, 419-422.

Gold, P. E. (1992). Modulation of memory processing: enhancement of memory in rodents and humans. In L.R.Squire & N. Butters (Eds.), Neuropsychology of Memory (2 ed., pp. 402-414). New York: The Guilford Press.

Gold, P. E. (1995). Role of glucose in regulating the brain and cognition. *American Journal of Clinical Nutrition, 61(suppl)*, 987S-995S.

Gold, P. E., Hankins, L., Edwards, R. M., & Chester, J. (1975). Memory interference and facilitation with posttrial amygdala stimulation: effect on memory varies with footshock level. *Brain Research, 86*, 509-513.

Gold, P. E. & McCarty, R. (1981). Plasma catecholamines: changes after footshock and seizure-producing frontal cortex stimulation. *Behavioral and Neural Biology, 31*, 247-260.

Gold, P. E. & McGaugh, J. L. (1975). A single-trace, two-process view of memory storage processes. In D.Deutsch & J. A. Deutsch (Eds.), Short-Term Memory (pp. 355-378). New York: Academic Press.

Gold, P. E. & van Buskirk, R. B. (1975). Facilitation of time-dependent memory processes with posttrial epinephrine injection. *Behavioral and Neural Biology, 31*, 247-260.

Gold, P. E., Vogt, J., & Hall, J. L. (1986). Glucose effects on memory: behavioral and pharmacological characteristics. *Behavioral and Neural Biology, 46*, 145-155.

Goldstein, M. L. (1960). Acquired drive strength as a joint function of shock intensity and number of acquisition trials. *Journal of Experimental Psychology, 60*, 349-358.

Goosens, K. A. & Maren, S. (2001). Contextual and auditory fear conditioning are mediated by the lateral, basal, and central amygdaloid nuclei in rats. *Learning and Memory, 8*, 148-155.

Guzowski, J. F. (2002). Insights into immediate-early gene function in hippocampal memory consolidation using antisense oligonucleotide and fluorescent imaging approaches. *Hippocampus, 12*, 86-104.

Halden, E. S. & Ross, G. R. T. (1967). Treatise on the passions of the soul (René Descartes; 1649). In E.S.Halden & G. R. T. Ross (Eds.), Philosophical Works of Descartes. Cambridge, UK: Cambridge University Press.

Hall, J. L. & Gold, P. E. (1986). The effects of training, epinephrine, and glucose injections on plasma glucose levels in rats. *Behavioral and Neural Biology, 46*, 156-167.

Hall, J., Thomas, K. L., & Everitt, B. J. (2001). Fear memory retrieval induces CREB phosphorylation and Fos expression within the amygdala. *European Journal of Neuroscience, 13*, 1453-1458.

Harris, J. A. & Westbrook, R. F. (1995). Effects of benzodiazepine microinjection into the

amygdala or periacqueductal gray on the expression of conditioned fear and hypoalgesia in rats. *Behavioral Neuroscience, 109*, 295-304.

Hatfield, T. & McGaugh, J. L. (1999). Norepinephrine infused into the basolateral amygdala posttraining enhances retention in a spatial water maze task. *Neurobiology of Learning and Memory, 71*, 232-239.

Hauber, W. & Schmidt, W. J. (1994). Differential effects of lesions of the dorsomedial and dorsolateral caudate-putamen on reaction time performance in rats. *Behavioural Brain Research, 60*, 211-215.

Hebb, D. O. (1946). On the nature of fear. *The Psychological Review, 53*, 259-276.

Hebb, D. O. (1955). Drives and the C.N.S. (conceptual nervous system). *The Psychological Review, 62*, 243-254.

Helmstetter, F. J. (1992). Contribution of the amygdala to learning and performance of conditional fear. *Physiology and Behavior, 51*, 1271-1276.

Helmstetter, F. J. (1993). Stress-induced hypoalgesia and defensive freezing are attenuated by application of diazepam to the amygdala. *Pharmacology, Biochemistry, and Behavior, 44*, 433-438.

Helmstetter, F. J. & Bellgowan, P. S. (1994). Effects of muscimol applied to the basolateral amygdala on acquisition and expression of contextual fear conditioning in rats. *Behavioral Neuroscience, 108*, 1005-1009.

Hess, U. S., Gall, C. M., Granger, R., & Lynch, G. (1997). Differential patterns of *c-fos* mRNA expression in amygdala during successive stages of odor discrimination learning. *Learning and Memory, 4*, 262-283.

Holahan, M. R. & White, N. M. (2001). Two-process learning theory and multiple memory systems. *Society for Neuroscience Abstracts, 27* [743.2].

Holahan, M. R. & White, N. M. (2002). Conditioned memory modulation, freezing, and avoidance as measures of amygdala-mediated conditioned fear. *Neurobiology of Learning and Memory, 77*, 250-275.

Holahan, M. R. & White, N. M. (2003). Effect of muscimol inactivation of the basolateral or central amygdala on shock-conditioned responses. *Annals of the New York Academy of Sciences, 985*, 525-527.

Holstege, G., Meiners, L., & Tan, K. (1985). Projections of the bed nucleus of the stria terminalis to the mesencephalon, pons, and medulla oblongata in the cat. *Experimental Brain Research, 58*, 370-391.

Hume, D. (2000). A treatise of human nature. Oxford: Oxford University Press.

Introini-Collison, I., Dalmaz, C., & McGaugh, J. L. (1996). Amygdala b-noradrenergic influences on memory storage involve cholinergic activation. *Neurobiology of Learning and Memory, 65*, 57-64.

Isaacson, R. L., Douglas, R. J., & Moore, R. Y. (1961). The effect of radical hippocampal ablation on acquisition of avoidance response. *Journal of Comparative and Physiological Psychology, 54*, 625-628.

Izquierdo, I., Da Cunha, C., Huang, C. H., & Walz, R. (1990). Post-training down-regulation of memory consolidation by a GABA-A mechanism in the amygdala modulated by endogenous benzodiazepines. *Behavioral and Neural Biology, 54*, 105-109.

James, W. (1884). What is an emotion? *Mind, 9*, 188-205.

James, W. (1890). Principles of psychology. New York: Henry Holt and Co.

Jellestad, F. K. & Cabrera, I. G. (1986). Exploration and avoidance learning after ibotenic acid and radio frequency lesions in the rat amygdala. *Behavioral and Neural Biology, 46*, 196-215.

Jodar, L., Takahashi, M., & Kaneto, H. (1996). FS stress induces long-lasting memory facilitation: involvement of cholinergic pathways. *Pharmacology, Biochemistry, and Behavior, 53*, 735-740.

Johnson, F. N. & Waite, K. (1971). Apparent delayed enhancement of memory following post-trial methylamphetamine HCl. *Experentia, 27*, 1316-1317.

Johnson, J. D., O'Connor, K. A., Deak, T., Spencer, R. L., Watkins, L. R., & Maier, S. F. (2002). Prior stress exposure primes the HPA axis. *Psychoneuroendocrinology, 27*, 353-365.

Kaczmarek, L. (1993). Molecular biology of vertebrate learning: is *c-fos* a new beginning? *Journal of Neuroscience Research, 34*, 377-381.

Kaczmarek, L. & Chaudhuri, A. (1997). Sensory regulation of immediate-early gene expression in mammalian visual cortex: implications for functional mapping and neural

plasticity. *Brain Research Reviews, 23*, 237-256.

Kaczmarek, L. & Nikolaev, E. (1990). C-Fos protooncogene expression and neuronal plasticity. *Acta Neurobiologiae Experimentalis, 50*, 173-179.

Kant, I. (1997). Lectures on metaphysics. Cambridge, UK: Cambridge University Press.

Keehn, J. D. (1967). Running and bar pressing as avoidance responses. *Psychological Reports, 20*, 591-602.

Keith, J. R. & Rudy, J. W. (1990). Why NMDA-receptor-dependent long-term potentiation may or may not be a mechanism of learning and memory: reappraisal of the NMDA-receptor blockade strategy. *Psychobiology, 18*, 251-257.

Kelley, A. E., Domesick, V. B., & Nauta, W. J. H. (1982). The amygdalostriatal projection in the rat - an anatomical study by anterograde and retrograde tracing methods. *Neuroscience, 7*, 615-630.

Kelly, P. H. (1977). Drug-induced motor behavior. In L.L.Iverson & S. D. Iversen (Eds.), Handbook of psychopharmacology. New York: Plenum Press.

Kesner, R. P. & Conner, H. S. (1974). Effects of electrical stimulation of limbic system and midbrain reticular formation upon short- and long-term memory. *Physiology and Behavior, 12*, 5-12.

Kesner, R. P. & Gilbert, P. E. (2001). Process-oriented view of amygdala and hippocampus. Mediation of reward value and spatial location information. In P.E.Gold & W. T. Greenough (Eds.), Memory Consolidation: Essays in Honor of James L. McGaugh (pp. 249-273). Washington, D.C.: American Psychological Association.

Kiernan, M. J. & Westbrook, R. F. (1993). Effects of exposure to a to-be-shocked environment upon the rat's freezing response: evidence for facilitation, latent inhibition, and perceptual learning. *The Quarterly Journal of Experimental Psychology, 46B*, 271-288.

Kiernan, M. J., Westbrook, R. F., & Cranney, J. (1995). Immediate shock, passive avoidance, and potentiated startle: implications for the unconditioned response to shock. *Animal Learning and Behavior, 23*, 22-30.

Killcross, S., Robbins, T. W., & Everitt, B. J. (1997). Different types of fear-conditioned behaviour mediated by separate nuclei within the amygdala. *Nature, 388*, 377-380.

Kim, J. J. & Fanselow, M. S. (1992). Modality-specific retrograde amnesia of fear. *Science, 256*, 675-677.

Kim, J. J., Rison, R. A., & Fanselow, M. S. (1993). Effects of amygdala, hippocampus, and periacqueductal gray lesions on short- and long-term contextual fear. *Behavioral Neuroscience, 107*, 1093-1098.

Kim, M. & Davis, M. (1993). Lack of temporal gradient of retrograde amnesia in rats with amygdala lesions assessed with fear-potentiated startle paradigm. *Behavioral Neuroscience, 107*, 1088-1092.

Kirkby, R. J. & Kimble, D. P. (1968). Avoidance and escape behavior following striatal lesions in the rat. *Experimental Neurology, 20*, 215-227.

Kirkby, R. J. & Polgar, S. (1974). Active avoidance in the laboratory rat following lesions of the dorsal or ventral caudate nucleus. *Physiological Psychology, 2*, 301-306.

Korte, S. M., Bouws, G. A. H., Koolhaas, J. M., & Bohus, B. (1992). Neuroendocrine and behavioral responses during conditioned active and passive behavior inthe defensive burying/probe avoidance paradigm: effects of ipsapirone. *Physiology and Behavior, 52*, 355-361.

Korte, S. M., Buwalda, B., Bouws, G. A. H., Koolhaas, J. M., Maes, F. W., & Bohus, B. (1992). Conditioned neuroendocrine and cardiovascular stress responsiveness accompanying behavioral passivity and activity in aged and in young rats. *Physiology and Behavior, 51*, 815-822.

Krettek, J. E. & Price, J. L. (1978b). A description of the amygdaloid complex in the rat and cat with observations on intra-amygdaloid axonal connections. *Journal of Comparative Neurology, 178*, 255-280.

Krettek, J. E. & Price, J. L. (1978a). Amygdaloid projections to subcortical structures within the basal forebrain and brainstem in the rat and cat. *Journal of Comparative Neurology, 178*, 225-253.

Kumar, R. (1970). Effects of fear on exploratory behaviour in rats. *Quarterly Journal of Experimental Psychology, 22*, 205-214.

Lanahan, A. & Worley, P. F. (1998). Immediate-early genes and synaptic function. *Neurobiology of Learning and Memory, 70*, 37-43.

Lashley, K. S. (1917). The effects of strychnine and caffeine upon the rate of learning. *Psychobiology, 1*, 141-170.

Lazarus, R. S. (1991). Progress on a cognitive-motivational-relational theory of emotion. *American Psychologist, 46*, 819-834.

Lechner, H. A., Squire, L. R., & Byrne, J. H. (1999). 100 years of consolidation - remembering Müller and Pilzecker. *Learning and Memory, 6*, 77-87.

LeDoux, J. (1998). Fear and the brain: where have we been, and where are we going? *Biological Psychiatry, 44*, 1229-1238.

LeDoux, J. (2000). The amygdala and emotion: a view through fear. In J.P.Aggleton (Ed.), The Amygdala: Second Edition: A Functional Analysis (2 ed., pp. 289-310). Oxford: Oxford University Press.

LeDoux, J. E. (1993). Emotional memory systems in the brain. *Behavioural Brain Research, 58*, 69-79.

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

LeDoux, J. E. (1996). Emotional networks and motor control: a fearful view. In G.Holstege, R. Bandler, & C. B. Saper (Eds.), Progress in Brain Research (pp. 437-446). Elsevier Science.

LeDoux, J. E., Cicchetti, P., Xagoraris, A., & Romanski, L. M. (1990). The lateral amygdaloid nucleus: sensory interface of the amygdala in fear conditioning. *The Journal of Neuroscience, 10*, 1062-1069.

LeDoux, J. E., Iwata, J., Cicchetti, P., & Reiss, D. J. (1988). Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlate of conditioned fear. *The Journal of Neuroscience, 8*, 2517-2529.

Lee, H. J., Choi, J.-S., Brown, T. H., & Kim, J. J. (2001). Amygdalar NMDA receptors are critical for the expression of multiple conditioned fear responses. *The Journal of Neuroscience, 21*, 4116-4124.

Lee, Y., Walker, D. L., & Davis, M. (1996). Lack of a temporal gradient of retrograde amnesia following NMDA-induced lesions of the basolateral amygdala assessed with the fear-potentiated startle paradigm. *Behavioral Neuroscience, 110*, 836-839.

Liang, K. C. & Lee, H. Y. (1988). Intra-amygdala injections of corticotropin releasing factor facilitate inhibitory avoidance learning and reduce exploratory behavior in rats. *Psychopharmacology, 96* , 232-236.

Liang, K. C. & McGaugh, J. L. (1983a). Lesions of the stria terminalis attenuate the amnestic effect of amygdaloid stimulation on avoidance responses. *Brain Research, 274*, 309-318.

Liang, K. C. & McGaugh, J. L. (1983b). Lesions of the stria terminalis attenuate the enhancing effect of post-training epinephrine on retention of an inhibitory avoidance response. *Behavioural Brain Research, 9*, 49-58.

Liang, K. C., McGaugh, J. L., Martinez, J. L., Jensen, R. A., Vasquez, B. J., & Messing, R. B. (1982). Post-training amygdaloid lesions impair retention of an inhibitory avoidance response. *Behavioural Brain Research* , *4*, 237-249.

Liang, K. C., McGaugh, J. L., & Yao, H.-Y. (1990). Involvement of amygdala pathways in the influence of post-training intra-amygdala norepinephrine and peripheral epinephrine on memory storage. *Brain Research, 508*, 225-233.

Locke, J. (1979). An essay concerning human understanding. Oxford: Clarendon Press.

Lu, K.-T., Walker, D. L., & Davis, M. (2001). Mitogen-activated protein kinase cascade in the basolateral nucleus of amygdala is involved in extinction of fear-potentiated startle. *The Journal of Neuroscience, 21*, 1-5.

Lubow, R. E. (1965). Latent inhibition: effects of frequency of nonreinforced preexposure of the CS. *Journal of Comparative and Physiological Psychology, 60*, 454-457.

Lubow, R. E., Markman, R. E., & Allen, J. (1968). Latent inhibition and classical conditioning of the rabbit pinna response. *Journal of Comparative and Physiological Psychology, 66*, 688-694.

Lubow, R. E. & Moore, A. U. (1959). Latent inhibition: the effect of nonreinforced pre-exposure to the conditional stimulus. *Journal of Comparative and Physiological Psychology, 52*, 415-419.

Mackenna, S. (1991). Plotinus: The Enneads. London: Penguin.

Malenka, R. C. & Nicoll, R. A. (1999). Long-term potentiation - a decade of progress? *Science, 285*, 1870-1874.

Maren, S. (1998). Overtraining does not mitigate contextual fear conditioning deficits

produced by neurotoxic lesions of the basolateral amygdala. *The Journal of Neuroscience*, *18*, 3088-3097.

Maren, S. (1999). Neurotoxic basolateral amygdala lesions impair learning and memory but not the performance of conditional fear in rats. *The Journal of Neuroscience*, *19*, 8696-8703.

Maren, S. (2001b). Is there savings for Pavlovian fear conditioning after neurotoxic basolateral amygdala lesions in rats? *Neurobiology of Learning and Memory*, *76*, 268-283.

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

Maren, S. (2003). The amygdala, synaptic plasticity, and fear memory. *Annals of the New York Academy of Sciences*, *985* , 106-113.

Maren, S., Aharonov, G., Stote, D. L., & Fanselow, M. S. (1996). *N*-Methyl-D-Aspartate receptors in the basolateral amygdala are required for both acquisition and expression of conditional fear in rats. *Behavioral Neuroscience*, *110*, 1365-1374.

McAllister, W. R. & McAllister, D. E. (1962). Role of CS and of apparatus cues in the measurement of acquired fear. *Psychological Reports*, *11*, 749-756.

McAllister, W. R. & McAllister, D. E. (1971). Behavioral measurement of conditioned fear. In F.R.Brush (Ed.), Aversive Conditioning and Learning (pp. 105-179). New York: Academic Press.

McAllister, W. R. & McAllister, D. E. (1995). Two-factor fear theory: implications for understanding anxiety-based clinical phenomena. In W.O'Donohue & L. Krasner (Eds.), Theories of Behavior Therapy: Exploring Behavior Change (pp. 145-171). Washington, D.C: American Psychological Association.

McDonald, A. J. (1991b). Organization of amydaloid projections to the prefrontal cortex and associated striatum in the rat. *Neuroscience* , *44*, 1-14.

McDonald, A. J. (1991a). Topographical organization of amygdaloid projections to the caudatoputamen, nucleus accumbens, and related striatal-like areas of the rat brain. *Neuroscience*, *44*, 15-33.

McGaugh, J. L. (1966). Time-dependent processes in memory storage. *Science*, *153*, 1351-

1358.

McGaugh, J. L. (1989). Dissociating learning and performance: drug and hormone enhancement of memory storage. *Brain Research Bulletin, 23*, 339-345.

McGaugh, J. L. (2000). Memory - a century of consolidation. *Science, 287*, 248-251.

McGaugh, J. L. (2002). Memory consolidation and the amygdala: a systems perspective. *Trends in Neurosciences, 25*, 456-561.

McGaugh, J. L. & Cahill, L. (1997). Interaction of neuromodulatory systems in modulating memory storage. *Behavioural Brain Research, 83*, 31-38.

McGaugh, J. L., Cahill, L., Ferry, B., & Roozendaal, B. (2000). Brain systems and the regulation of memory consolidation. In J.J.Bolhuis (Ed.), Brain, Perception, Memory: Advances in Cognitive Neuroscience (pp. 233-251). Oxford: Oxford University Press.

McGaugh, J. L., Cahill, L., & Roozendaal, B. (1996). Involvement of the amygdala in memory storage: interaction with other brain systems. *Proceedings of the National Academy of Sciences of the United States of America, 93*, 13508-13514.

McGaugh, J. L. & Herz, M. J. (1972). Memory consolidation. San Francisco: Albion.

McGaugh, J. L., Introini-Collison, I., Juler, R. G., & Izquierdo, I. (1986). Stria terminalis lesions attenuate the effects of posttraining naloxone and beta-endorphin on retention. *Behavioral Neuroscience, 100*, 839-844.

McGaugh, J. L. & Petrinovich, L. (1959). The effect of strychnine sulphate on maze-learning. *American Journal of Psychology, 72*, 99-102.

McGaugh, J. L. & Petrinovich, L. (1963). Comments concerning the basis of learning enhancement with central nervous system stimulants. *Psychological Reports, 12*, 211-214.

McGaugh, J. L., Thomson, C. W., Westbrook, W. H., & Hudspeth, W. J. (1962). A further study of learning facilitation with strychnine sulphate. *Psychopharmacologia, 3*, 352-360.

McGaugh, J. L., Westbrook, W. H., & Thomson, C. W. (1962). Facilitation of maze learning with posttrial injections of 5-7-dipheyl-1-3-diazadamantan-6-ol (1757 I.S.). *Journal of Comparative and Physiological Psychology, 55*, 710-713.

McNish, K. A., Gewirtz, J. C., & Davis, M. (1997). Evidence of contextual fear after lesions of the hippocampus: a disruption of freezing but not fear-potentiated startle. *The Journal*

of Neuroscience, *17*, 9353-9360.

Messier, C. & White, N. M. (1984). Contingent and non-contingent actions of sucrose and saccharin reinforcers: effects on taste preference and memory. *Physiology and Behavior*, *32*, 195-203.

Messier, C. & White, N. M. (1987). Memory improvement by glucose, fructose, and two glucose analogs: a possible effect on peripheral glucose transport. *Behavioral and Neural Biology*, *48*, 104-127.

Milanovic, S., Radulovic, J., Laban, O., Stiedl, O., Henn, F., & Spiess, J. (1998). Production of the Fos protein after contextual fear conditioning of C57BL/6N mice. *Brain Research*, *784*, 37-47.

Miller, N. E. (1948). Studies of fear as an acquirable drive: I. Fear as motivation and fear-reduction as reinforcement in the learning of new responses. *Journal of Experimental Psychology*, *38*, 89-101.

Milner, P. M. (1957). The cell assembly: mark II. *Psychological Review*, *64*, 242-252.

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

Morán, M. A., Mufson, E. J., & Mesulam, M.-M. (1987). Neural inputs into the temporalpolar cortex of the rhesus monkey. *Journal of Comparative Neurology*, *256*, 88-103.

Morgan, J. I. & Curran, T. (1991). Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes *fos* and *jun*. *Annual Review of Neuroscience* , *14*, 421-451.

Mowrer, O. H. (1939). A stimulus-response analysis of anxiety and its role as a reinforcing agent. *Psychological Review*, *46*, 553-565.

Mowrer, O. H. (1947). On the dual nature of learning -- A re-interpretation of "conditioning" and "problem-solving". *Harvard Educational Review*, *17*, 102-148.

Mowrer, O. H. & Lamoreaux, R. R. (1946). Fear as an intervening variable in avoidance conditioning. *Journal of Comparative Psychology*, *39*, 29-50.

Mowrer, O. H. & Miller, N. E. (1942). A multi-purpose learning-demonstration apparatus.

Journal of Experimental Psychology, 30, 163-170.

Muller, J., Corodimas, K. P., Fridel, Z., & LeDoux, J. E. (1997). Functional inactivation of the lateral and basal nuclei of the amygdala by muscimol infusion prevents fear conditioning to an explicit conditioned stimulus and to contextual stimuli. *Behavioral Neuroscience, 111*, 683-691.

Nader, K. (2003). Memory traces unbound. *Trends in Neurosciences, 26*, 65-72.

Nader, K., Majidishad, P., Amorapanth, P., & LeDoux, J. E. (2001). Damage to the lateral and central, but not other, amygdaloid nuclei prevents the acquisition of auditory fear conditioning. *Learning and Memory, 8*, 156-163.

Nader, K., Schafe, G. E., & LeDoux, J. E. (2000a). Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. *Nature, 406*, 722-726.

Nader, K., Schafe, G. E., & LeDoux, J. E. (2000b). The labile nature of consolidation theory. *Nature Reviews: Neuroscience, 1*, 216-219.

Nguyen, K. T., Deak, T., Owens, S. M., Kohno, T., Fleshner, M., Watkins, L. R., & Maier, S. F. (1998). Exposure to acute stess indues brain interlukin-1beta protein in the rat. *The Journal of Neuroscience, 18*, 2239-2246.

Olton, D. S. (1973). Shock-motivated avoidance and the analysis of behavior. *Psychological Bulletin, 79*, 243-251.

Packard, M. G., Cahill, L., & McGaugh, J. L. (1994). Amygdala modulation of hippocampal-dependent and caudate nucleus-dependent memory processes. *Proceedings of the National Academy of Sciences of the United States of America, 91*, 8477-8481.

Packard, M. G. & Chen, S. A. (1999). The basolateral amygala is a cofactor in memory enhancement produced by intrahippocampal glutamate injections. *Psychobiology, 27*, 377-385.

Packard, M. G. & Teather, L. A. (1998). Amygdala modulation of multiple memory systems: hippocampus and caudate-putamen. *Neurobiology of Learning and Memory, 69*, 163-203.

Parent, A., Mackey, A., & DeBellefeuille, L. (1983). The subcortical afferents to caudate nucleus and putamen in primate: a fluorescence retrograde double labeling study. *Neuroscience, 10*, 1137-1150.

Parent, M. B., Avila, E., & McGaugh, J. L. (1995). Footshock facilitates the expression of aversively motivated memory in rats given post-training amygdala basolateral complex lesions. *Brain Research, 676*, 235-244.

Parent, M. B. & McGaugh, J. L. (1994). Posttraining infusion of lidocaine into the amygdala basolateral complex impairs retention of inhibitory avoidance training. *Brain Research, 661*, 97-103.

Parent, M. B., Quirarte, G. L., Cahill, L., & McGaugh, J. L. (1995). Spared retention of inhibitory avoidance learning after posttraining amygdala lesions. *Behavioral Neuroscience, 109*, 803-807.

Parent, M. B., Tomaz, C., & McGaugh, J. L. (1992). Increased training in an aversively motivated task attenuates the memory-impairing effects of posttraining *N*-Methyl-D-Aspartate-induced amygdala lesions. *Behavioral Neuroscience, 106*, 789-797.

Parent, M. B., West, M., & McGaugh, J. L. (1994). Memory of rats with amygdala lesions induced 30 days after footshock-motivated escape training reflects degree of original training. *Behavioral Neuroscience, 108*, 1080-1087.

Paré, D., Collins, D. R., & Pelletier, J. G. (2002). Amygdala oscilliations and the consolidation of emotional memories. *Trends in Neurosciences, 6*, 306-314.

Pavlov, I. P. (1927). Conditioned reflexes. Oxford: Oxford University Press.

Paxinos, G. & Watson, C. (1998). The rat brain atlas in stereotaxic coordinates. (4 ed.) New York: Academic Press.

Perez, L. & Lysle, D. T. (1995). Corticotropin-releasing hormone is involved in conditioned stimulus-induced reduction of natural killer cell activity but not in conditioned alterations in cytokine production or proliferation responses. *Journal of Neuroimmunology, 63*, 1-8.

Pezzone, M. A., Lee, W.-S., Hoffman, G. E., & Rabin, B. S. (1992). Induction of c--Fos immunoreactivity in the rat forebrain by conditioned and unconditioned aversive stimuli. *Brain Research, 597*, 41-50.

Phillips, R. G. & LeDoux, J. E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behavioral Neuroscience, 106*, 274-285.

Pitkänen, A. (2000). Connectivity of the rat amygdaloid complex. In J.P.Aggleton (Ed.), The

Amygdala: A Functional Analysis. (2 ed., pp. 31-115). Oxford: Oxford University Press.

Poremba, A. & Gabriel, M. (1997). Amygdalar lesions block discriminative avoidance learning and cingulothalamic training-induced neuronal plasticity in rabbits. *The Journal of Neuroscience, 17*, 5237-5244.

Power, A. E., Roozendaal, B., & McGaugh, J. L. (2000). Glucocorticoid enhancement of memory consolidation in the rat is blocked by muscarinic receptor antagonism in the basolateral amygdala. *European Journal of Neuroscience, 12*, 3481-3487.

Price, J. L. & Amaral, D. G. (1981). An autoradiographic study of the projections of the central nucleus of the monkey amygdala. *The Journal of Neuroscience, 1*, 1242-1259.

Quirarte, G. L., Roozendaal, B., & McGaugh, J. L. (1997). Glucocorticoid enhancement of memory storage involves noradrenergic activation in the basolateral amygdala. *Proceedings of the National Academy of Sciences of the United States of America, 94*, 14048-14053.

Radulovic, J., Kammermeier, J., & Spiess, J. (1998). Relationship between Fos production and classical fear conditioning: effects of novelty, latent inhibition, and unconditioned stimulus preexposure. *The Journal of Neuroscience, 18*, 7452-7461.

Rescorla, R. A. (1988). Behavioral studies of Pavlovian conditioning. *Annual Review of Neuroscience, 11*, 329-352.

Rescorla, R. A. (1996). Preservation of Pavlovian associations through extinction. *Quarterly Journal of Experimental Psychology: Section B - Comparative and Physiological Psychology, 49*, 245-258.

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

Roesler, R., Roozendaal, B., & McGaugh, J. L. (2002). Basolateral amygdala lesions block the memory-enhancing effect of 8-Br-cAMP infused into the entorhinal cortex of rats after training. *European Journal of Neuroscience, 15*, 905-910.

Romanski, L. M. & LeDoux, J. E. (1992). Equipotentiality of thalamo-amygdala and thalamo-cortico-amygdala ciruits in auditory fear conditioning. *The Journal of Neuroscience, 12*, 4501-4509.

Roozendaal, B., Brunson, K. L., Holloway, B. L., McGaugh, J. L., & Baram, T. Z. (2002). Involvement of stress-released corticotropin-releasing hormone in the basolateral amygdala in regulating memory consolidation. *Proceedings of the National Academy of Sciences of the United States of America, 99*, 13908-13913.

Roozendaal, B. & McGaugh, J. L. (1996). Amygdaloid nuclei lesions differentially affect glucocorticoid-induced memory enhancement in an inhibitory avoidance task. *Neurobiology of Learning and Memory, 65*, 1-8.

Roozendaal, B. & McGaugh, J. L. (1997b). Basolateral amygdala lesions block the memory-enhancing effect of glucocorticoid administration in the dorsal hippocampus of rats. *European Journal of Neuroscience, 9*, 76-83.

Roozendaal, B. & McGaugh, J. L. (1997a). Glucocorticoid receptor agonist and antagonist administration into the basolateral but not central amygdala modulates memory storage. *Neurobiology of Learning and Memory, 67*, 176-179.

Roozendaal, B., Nguyen, B. T., Power, A. E., & McGaugh, J. L. (1999). Basolateral amygdala noradrenergic influence enables enhancement of memory consolidation induced by hippocampal glucocorticoid receptor activation. *Proceedings of the National Academy of Sciences of the United States of America, 96*, 11642-11647.

Rose, S. P. R. (1991). How chicks make memories: the cellular cascade from *c-fos* to dendritic remodelling. *Trends in Neurosciences, 14*, 390-397.

Rose, S. P. R. (1996). Cell adhesion molecules and the transition from short- to long-term memory. *Journal of Physiology (Paris), 90*, 387-391.

Rose, S. P. R. (2000). God's organism? The chick as a model system for memory studies. *Learning and Memory, 7*, 1-17.

Rosen, J. B., Fanselow, M. S., Young, S. L., Sitcoske, M., & Maren, S. (1998). Immediate-early gene expression in the amygdala following footshock stress and contextual fear conditioning. *Brain Research, 796*, 132-142.

Sacchetti, B., Lorenzini, C. A., Baldi, E., Tassoni, G., & Bucherelli, C. (1999). Memorization of contextual and CS conditioned fear response (freezing) in a one-trial acquisition paradigm. *Archives Italiennes de Biologie, 137*, 235-248.

Sander, G., Oberling, P., Silveira, M. C., Di Scala, G., Rocha, B., Bagri, A., & Depoortere,

R. (1993). What brain structures are active during emotions? Effects of brain stimulation elicited aversion on *c-fos* immunoreactivity and behavior. *Behavioural Brain Research, 58*, 9-18.

Sanes, J. R. & Lichtman, J. W. (1999). Can molecules explain long-term potentiation? *Nature Neuroscience, 2*, 597-604.

Sarter, M. & Markowitsch, H. J. (1985). Involvement of the amygdala in learning and memory: a critical review, with emphasis on anatomical relations. *Behavioral Neuroscience, 99*, 342-380.

Savonenko, A., Filipkowski, R. K., Werka, T., Zielinski, K., & Kaczmarek, L. (1999). Defensive conditioning-related functional heterogeneity among nuclei of the rat amygdala revealed by c-Fos mapping. *Neuroscience, 94*, 723-733.

Schafe, G. E., Atkins, C. M., Swank, M. W., Bauer, E. P., Sweatt, J. D., & LeDoux, J. E. (2000). Activation of ERK/MAP kinase in the amygdala is required for memory consolidation of Pavlovian fear conditioning. *The Journal of Neuroscience, 20*, 8177-8187.

Schafe, G. E. & LeDoux, J. E. (2000). Memory consolidation of auditory Pavlovian fear conditioning requires protein synthesis and protein kinase A in the amygdala. *The Journal of Neuroscience, 20*, 1-5.

Selden, N. R. W., Everitt, B. J., Jarrard, L. E., & Robbins, T. W. (1991). Complementary roles for the amygdala and hippocampus in aversive conditioning to explicit and contextual cues. *Neuroscience, 42*, 335-350.

Sheng, M. & Greenberg, M. E. (1990). The regulation and function of *c-fos* and other immediate early genes in the nervous system. *Neuron, 4*, 477-485.

Sheng, M., McFadden, G., & Greenberg, M. E. (1990). Membrane depolarization and calcium induce *c-fos* transcription via phosphorylation of transcription factor CREB. *Neuron, 4*, 571-582.

Shin, C., McNamara, J., Morgan, J. I., & Curran, T. (1990). Induction of c-fos mRNA expression by after-discharge in the hippocampus of naive and kindled rats. *Journal of Neurochemistry, 55*, 1050-1055.

Shurin, M. R., Kusnecov, A. W., Riechman, S. E., & Rabin, B. S. (1995). Effect of a

conditioned aversive stimulus on the immune response in three strains of rats. *Psychoneuroendocrinology, 20*, 837-849.

Sidman, M. (1962b). An adjusting avoidance schedule. *Journal of the Experimental Analysis of Behavior, 5*, 271-277.

Sidman, M. (1962a). Classical avoidance without a warning stimulus. *Journal of the Experimental Analysis of Behavior, 5*, 97-104.

Smith, D. M., Monteverde, J., Schwartz, E., Freeman, J. H., & Gabriel, M. (2001). Lesions of the central nucleus of the amygdala: discriminative avoidance learning, discriminative approach learning, and cingulothalamic training-induced neuronal activity. *Neurobiology of Learning and Memory, 76*, 403-425.

Sotty, F., Sander, G., & Gosselin, O. (1996). Latent inhibition in conditioned emotional response: *c-fos* immunolabelling evidence for brain areas involved in the rat. *Brain Research, 737*, 243-254.

Sovran, P. (1994). *A behavioural and anatomical investigation of amygdaloid mediation of affective memory*. Master's Thesis; McGill University.

Steffens, A. B. (1969). Rapid absorption of glucose in the intestinal tract of the rat after ingestion of a meal. *Physiology and Behavior, 4*, 829-832.

Steffens, A. B. (1970). Plasma insulin content in relation to blood glucose level and meal pattern in the normal and hypothalamic hyperphagic rat. *Physiology and Behavior, 5*, 147-151.

Stork, O., Stork, S., Pape, H.-C., & Obata, K. (2001). Identification of genes expressed in the amygdala during the formation of fear memory. *Learning and Memory, 8*, 209-219.

Turner, B. H., Mishkin, M., & Knapp, M. (1980). Organization of the amygdalopetal projections from modality-specific cortical association areas in the monkey. *Journal of Comparative Neurology, 191*, 515-543.

van Hoesen, G. W. (1981). The differential distribution, diversity, and sprouting of cortical projections to the amygdala in the rhesus monkey. In Y.Ben-Ari (Ed.), The Amygdaloid Complex (pp. 77-90). Amsterdam: Elsevier/North Holland Press.

Van Wimersma Greidanus, T. B., Croiset, G., Bakker, E., & Bouman, H. (1979). Amygdaloid lesions block the effect of neuropeptides (vasopressin, $ACTH_{4\text{-}10}$) on

avoidance behavior. *Physiology and Behavior, 22*, 291-295.

Vazdarjanova, A., Cahill, L., & McGaugh, J. L. (2001). Disrupting basolateral amygdala function impairs unconditioned freezing and avoidance in rats. *European Journal of Neuroscience, 14*, 709-718.

Vazdarjanova, A. & McGaugh, J. L. (1998). Basolateral amygdala is not critical for cognitive memory of contextual fear conditioning. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 15003-15007.

Vazdarjanova, A. & McGaugh, J. L. (1999). Basolateral amygdala is involved in modulating consolidation of memory for classical fear conditioning. *The Journal of Neuroscience, 19*, 6615-6622.

Vianna, M. R. M., Szapiro, G., McGaugh, J. L., Medina, J. H., & Izquierdo, I. (2001). Retrieval of memory for fear-motivated training initiates extinction requiring protein synthesis in the rat hippocampus. *Proceedings of the National Academy of Sciences of the United States of America, 98*, 12251-12254.

Viaud, M. D. & White, N. M. (1989). Dissociation of visual and olfactory conditioning in the neostriatum of rats. *Behavioural Brain Research, 32*, 31-42.

Wadenberg, M.-L. G. & Hicks, P. B. (1999). The conditioned avoidance response test re-evaluated: is ti a sensitive test for the detection of potentially atypical antipsychotics? *Neuroscience and Biobehavioral Reviews , 23*, 851-862.

Walker, D. L. & Davis, M. (2002). The role of amygdala glutamate receptors in fear learning, fear-potentiated startle, and extinction. *Pharmacology, Biochemistry, and Behavior, 71*, 379-392.

Walker, D. L., Toufexis, D. J., & Davis, M. (2003). Role of the bed nucleus of the stria terminalis versus amygdala in fear, stress, and anxiety. *European Journal of Pharmacology, 463*, 199-216.

Wallace, K. J. & Rosen, J. B. (2000). Predator odor as an unconditioned fear stimulus in rats: elicitation of freezing by trimethylthiazoline, a component of fox feces. *Behavioral Neuroscience, 114*, 912-922.

Westbrook, W. H. & McGaugh, J. L. (1964). Drug facilitation of latent learning. *Psychopharmacologia, 5*, 440-446.

White, N. M. (1998). Cognitive enhancement: an everyday event? *International Journal of Psychology, 33*, 95-105.

White, N. M. & Carr, G. D. (1985). The conditioned place preference is affected by two independent reinforcement processes. *Pharmacology, Biochemistry, and Behavior, 23*, 37-42.

White, N. M. & Legree, P. (1984). Effects of posttraining exposure to an aversive stimulus on retention. *Physiological Psychology, 12*, 233-236.

White, N. M. & McDonald, R. J. (2002). Multiple parallel memory systems in the brain of the rat. *Neurobiology of Learning and Memory, 77*, 125-184.

White, N. M. & Messier, C. (1988). Effects of adrenal demedullation on the conditioned emotional response and on the memory improving action of glucose. *Behavioral Neuroscience, 102*, 499-503.

White, N. M. & Salinas, J. A. (2003). Mnemonic functions of the dorsal striatum and hippocampus in aversive conditioning. *Behavioural Brain Research, 142*, 99-107.

Wilensky, A. E., Schafe, G. E., & LeDoux, J. E. (1999). Functional inactivation of the amygdala before but not after auditory fear conditioning prevents memory formation. *The Journal of Neuroscience, 19*, 1-5.

Wilensky, A. E., Schafe, G. E., & LeDoux, J. E. (2000). The amygdala modulates memory consolidation of fear-motivated inhibitory avoidance learning but not classical fear conditioning. *The Journal of Neuroscience, 20*, 7059-7066.

Williams, C. L., Men, D., Clayton, E. C., & Gold, P. E. (1998). Norepinephrine release in the amygdala after systemic injection of epinephrine or escapable footshock: contribution of the nucleus of the solitary tract. *Behavioral Neuroscience, 112*, 1414-1422.

Zangrossi, H. & File, S. E. (1992). Behavioral consequences in animals tests of anxiety and exploration of exposure to cat odor. *Brain Research Bulletin, 29*, 381-388.

Zangrossi, H. & File, S. E. (1994). Habituation and generalization of phobic responses to cat odor. *Brain Research Bulletin, 33*, 189-194.

Zeyl, D. J. (2000). Timaeus; Plato. Indianapolis: Hackett Publishing Company.

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Animal Use Protocol – Research

Guidelines for completing the form are available at www.mcgill.ca/fgsr/rgo/animal/

(For office use)
Protocol #: 1417
Investigator #: 157
Approval End Date: March 31, 2004
Facility Committee: SCI

☐ Pilot ☐ New Application ☒ Renewal of Protocol # 1417

Title (must match the title of the funding source application): Neural and Physiological Analysis of Mechanisms of Reinforcement

1. Investigator Data:

Principal Investigator:	Norman White	Office #:	6082
Department:	Psychology	Fax#:	4896
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2. Emergency Contacts: Two people must be designated to handle emergencies.

Name:	Michael Roberts	Work #:	398-6091	Emergency #:	985-5489
Name:	Norman White	Work #:	398-6082	Emergency #:	574-0544

3. Funding Source:

External ☒
Source (s): NSERC

Peer Reviewed: ☒ YES ☐ NO**

Status : ☒ Awarded ☐ Pending

Funding period: April 1, 2002 - March 31, 2006

Internal ☐
Source (s):

Peer Reviewed: ☐ YES ☐ NO**

Status: ☐ Awarded ☐ Pending

Funding period:

** All projects that have not been peer reviewed for scientific merit by the funding source require 2 Peer Review Forms to be completed . e.g. Projects funded from industrial sources. Peer Review Forms are available at www.mcgill.ca/fgsr/rgo/animal/

Proposed Start Date of Animal Use (d/m/y):

Expected Date of Completion of Animal Use or ongoing ☒

Investigator's Statement: The information in this application is exact and complete. I assure that all care and use of animals in this proposal will be in accordance with the guidelines and policies of the Canadian Council on Animal Care and those of McGill University. I shall request the Animal Care Committee's approval prior to any deviations from this protocol as approved. I understand that this approval is valid for one year and must be approved on an annual basis.

Principal Investigator: Norman White		Date: February 11, 2003
Approval Signatures		
Chair, Facility Animal Care Committee:		MAY 1 2003
University Veterinarian:		Date: 5/15/03
Chair, Ethics Subcommittee(as per UACC policy):		Date: May 15, 03
Approved Period for Animal Use	Beginning: April 1, 2003	Ending: MARCH 31, 2004

☐ This protocol has been approved with the modifications noted in Section 13.

4. **Research Personnel and Qualifications:** List the names of all individuals who will be in contact with animals in this study (including the Principal Investigator) and their employment classification (investigator, technician, research assistant, undergraduate/graduate student, fellow). Indicate any training received (e.g workshops, lectures, etc.). The PI certifies that all personnel listed here have suitable training and/or experience, or will be provided with the specific training which qualifies them to perform the procedures described in the protocol. Each person listed in this section must sign to indicate that s/he has read this protocol. (Space will expand as needed.)

Name	Classification	Training Information	Signature
Norman White	Professor		
Eric Stouffer	Graduate Student	4 years experience	
Michael Roberts	Graduate Student	Course in laboratory procedures at U of Man, 3 years experience	
Stephane Gaskin	Graduate Student	Courses in Lab Safety and Rodent Handling Procedures, Concordia U	

** If an undergraduate student is involved , the role of the student and the supervision received must be described.

5. **Summary (In language that will be understood by members of the general public)**

a) Rationale: Describe, in a short paragraph, the overall aim of the study and its potential benefit to human/animal health or to the advancement of scientific knowledge.

The overall object of this research project is to understand how certain types of events (reinforcers) control behavior. Reinforcers are events that have three kinds of effects on behavior: they elicit positive and negative affective states, they elicit approach or escape responses and they modulate memory. Understanding the neural basis of these actions will further both basic understanding of an important aspect of behavior control, and will also contribute to the solution of behavioral health problems such as drug addiction and obesity.

b) Specific Objectives of the Study: Summarize in point form the primary objectives of this study.

The aim of the experiments is to examine the roles of the different parts of the amygdala and its efferent projections in the effects of reinforcers on behavior. We will be testing the hypothesis that different parts of the amygdala and different projections mediate each of the three effects described. We will also be including other brain areas, the hippocampus and caudate nucleus in our studies of these roles of reinforcers.

c) Progress Report: If this is a renewal of an ongoing project, **briefly** summarize what was accomplished during the prior approval period and indicate if and how the current goals differ from those in the original application.

In the past year we have made progress on experiments examining the memory improving effects of reinforcers, showing that they can be produced by both aversive and rewarding events. During the coming year we plan to extend this work to compare the brain mechanisms that produce these two effects.

d) Summary of Procedures for Animal Use Report to the CCAC : Using **key words,** describe the procedures used (e.g. anaesthesia, breeding colony, injection IP, gavage, drug administration, major survival surgery, euthanasia by exsanguination, behavioural studies). Refer to Appendix 1of the Guidelines for a more complete list of suggested key words.

stereotaxic surgery, systemic and intracranial injections of drugs, behavioral testing, food deprivation, footshock

6. **Animals To Be Used**

a) **Purpose of Animal Use** (Check one):

1. ☒ Studies of a fundamental nature/basic research
2. ☐ Studies for medical purposes relating to human/animal diseases/disorders
3. ☐ Regulatory testing
4. ☐ Development of products/appliances for human/veterinary medicine

b) Will the project involve breeding animals? NO☒ YES ☐

Will the project involve the generation of genetically altered animals? NO ☒ YES ☐

Will field studies be conducted? NO ☒ YES ☐