UNIVERSITY OF CALIFORNIA, SAN DIEGO

The Role of the Basolateral Amygdala in Affective Associative Learning, Arousal and Adaptation.

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in Cognitive Science

by

Irina Y Merzlyak

Committee in charge:

Professor Andrea A. Chiba, Chair
Professor George Koob
Professor Jaime Pineda
Professor Morton P. Printz
Professor Martin Sereno
Professor Lisa Stefanacci

2006

The dissertation of Irina Y Merzlyak is approved, and it is acceptable in quality and form for publication on microfilm:

Chair

University of California, San Diego

2006

Папе, маме, Анечке

TABLE OF CONTENTS

LIST OF FIGURES

ACKNOWLEDGEMENTS

Andrea Chiba, Laleh Quinn, Morton Printz, Lisa Stefanacci, Martin Sereno, Jaime Pineda, George Koob, Rebecca Jaworski, Lisa Conti, Anthony Nguyen, Lara Maria Rangel, Chris Cordova, Allyson Bell, Allister Liao, David Santucci, Graham Pechenik, Karen Blais, Sharis Nazarian, Michal Zeituni, Elizabeth Fuller, David Piccinati

My Parents: Yuri and Tanya Merzlyak, My sister: Anna Merzlyak, The Wichita crowd, The Chicago crowd whom I love dearly, Carrie Armel, Nadia Raizer, Roxanne Sweney, Nikolay Osadchiy, All of my dear friends from all over for their unwavering support!!, All of “our” cogsci class – the nth year, Garrisson Cottrell and the IGERT crowd, NSF and IGERT for funding this work and myself

CURRICULUM VITA

Education:

1994-1998	Northwestern University, Evanston, IL
1998	Bachelor of Arts, Northwestern University Major: Biological Sciences (concentration: Neurobiology) 2nd Major: Psychology
1998 - 2006	University of California at San Diego
2006	Ph.D., University of California at San Diego Field: Cognitive Science (concentration: Behavioral Neuroscience)

Laboratory Positions/Experience:

1999-2005

Cognitive neuroscience research in the laboratory of Dr. Chiba, Cognitive Science Department, UCSD.

1996-1998

Undergraduate independent research and Honors Thesis research in the neuroendocrinology and circadian rhythms laboratory of Dr. Turek, Northwestern University.

Laboratory Positions/Experience: (continued)

1996-1998

Laboratory Assistant in the Genetics and Molecular Biology Laboratory of Dr. Gaber, Northwestern University.

Teaching Experience:

Cognitive Science Department, UCSD

Fall, 1999; Fall, 2000; Winter 2004: *Neurobiology of Cognition*, CS17, Teaching Assistant (TA)

Winter, 1999: *Cognitive Neuroscience: Systems Neurobiology*, 107B, TA

Spring, 2000; Spring 2001: *Cog. Neuroscience: Neural Bases of Cognition*, 107C, TA

Spring, 1999; Winter 2001, 2003; Summer 2004: *Brain Disorders and Cognition*, CS172, TA

Fall 2003: *Cognitive Neuroscience: Systems Neurobiology*, 107A, TA

Spring, 2004: *Introduction to Cognitive Science*, COGS1, Instructor

Honors/Awards:

September, 2003 NSF Integrative Graduate Education and Research Traineeship (IGERT) Fellowship

2002-2003 Malcolm R. Stacey Memorial Scholarship

1999, 2004 Distinguished Teaching Assistant Award, Department of Cognitive Science, UCSD

1998-2002 UCSD Fellowship

June, 1998 Departmental Honors in Biological Sciences

Publications:

Bernard, DJ; Merzlyak, IY; Horton, TH; Turek, FW. Differential regulation of pituitary gonadotropin subunit messenger ribonucleic acid levels in photostimulated Siberian hamsters. *Biology of Reproduction*, 2000 Jan, 62(1):155-61.

Manuscripts in Preparation:

Quinn L.K., Merzlyak I.Y., Minces V., Chiba A.A. Neural Activity in the Rat Basolateral Amygdala Reflects the Acquired Motivational Significance of Visual Objects.

Merzlyak, IY and Chiba AA. Neural Mechanisms of Allostasis: A Comprehensive Review.

Posters:

I.Y. Merzlyak, L.K. Quinn, A.K. Bell, A.A. Chiba. Excitotoxic Lesions of Basolateral Amygdala in Rats Alter Affective Associative Learning of Visual Objects. 2003 Washington, DC: Society for Neuroscience, 2003.

A.A. Chiba, L.K. Quinn, I.Y. Merzlyak. Neural Activity in the Rat Basolateral Amygdala Reflects the Acquired Motivational Significance of Visual Objects. Program No. 284.8. *2002 Abstract Viewer/Itinerary Planner*. Washington, DC: Society for Neuroscience, 2002. Online.

Merzlyak, IY; Bernard, DJ; Turek, FW. Co-author and Presenter of poster: "Differential regulation of the gonadotropins in Siberian hamsters." *18th Annual Minisymposium on Reproductive Biology*, 1997.

Talks:

Data Blitz Presentation: BLA in Affective Associative Learning. Annual Conference on the Neurobiology of Learning and Memory, Park City, Utah, January 12, 2004.

Functional Neuroanatomy tutorial - Integrative Graduate Education and Research Traineeship (IGERT) Boot Camp, UCSD, September 15, 2004.

Functional Neuroanatomy tutorial - The Third International Conference on Development and Learning (ICDL'04) Developing Social Brains, UCSD, October 20, 2004.

ABSTRACT OF THE DISSERTATION

The Role of the Basolateral Amygdala in Affective Associative Learning, Arousal and Adaptation.

by

Irina Y Merzlyak
Doctor of Philosophy in Cognitive Science
University of California, San Diego, 2006
Professor Andrea A. Chiba, Chair

Affect is a highly adaptive and complex organismal state controlled by neural processes involved in all levels of brain function from autonomic reflexes to learning, memory, and complex behavioral outputs. One of the structures strongly implicated in affective processing is the basolateral amygdala.

The basolateral amygdala complex (BLA) has a well established and important role in learning the associations between neutral stimuli and rewards or punishments. BLA is also an intrinsic part of subcortical circuitry involved in reward and stress processing, including downstream projections to the brainstem autonomic nuclei. A set of studies have been conducted to examine the extent of involvement of the amygdala in several paradigms that encompass associative learning and behavior. First the effects of neurotoxic BLA lesions were tested during an associative learning task in which neutral visual objects were paired with either positive or aversive food pellets. Formation of preferences for the objects based on the associated affective value was correlated with behavioral outcomes during this task. In two different chapters of this thesis, the role of the BLA is explored in overt choice behaviors and in instrumental response learning. The relationship between learning behavior and autonomic responses was tested in a separate study designed to further elucidate the role of BLA in affect. Blood pressure was measured during contingent and non-contingent behavioral contexts. The results were indicative of the interaction between a role for the BLA in place preference and physiological response.

In this thesis a number of hypotheses on associative learning, affect, arousal and organismal function in the environment were tested and integrated. The findings presented here are in support of a theoretical construct emphasizing a pivotal role for the extended amygdala and related subcortical structures in associative learning and adaptation to the environment.

I

Introduction

Life in the ever changing world continually presents new challenges and unfamiliar obstacles. When faced with novel situations organisms can either fluidly evolve and adapt their actions or face unpleasant circumstances. This process of adaptation occurs on many distinct levels of organismal function. Depending on the presented obstacle, the analysis of the situation may happen on the level of perception, cognition, behavior, on various physiological levels, or, more likely, involve a combination of these functions unique to that obstacle.

The topics discussed in this thesis address how some of these functions, including behavior, physiology, and, potentially, cognitive processing, interact when an organism is faced with learning novel stimuli and situations. Some of the neural structures driving these adaptive learning responses are manipulated and explored with regard to their functional contribution.

The work presented here also includes a theoretical synthesis of what happens to the neural control of learning and responding when an organism fails to adapt successfully to the environment. The process of maladaptation to the external environment is explored with regard to the alterations imposed on the neural structures important for the proper regulation of cognitive, behavioral, and physiological functions.

1. Rationale and Theoretical Framework

One encounters external environmental stimuli constantly, some old, some novel. The process of evaluation of a neutral stimulus, or "appraisal", involves making an affective or evaluative judgment. Affective judgments entail making an association between a stimulus and its proximal hedonistic value. “Judgments” can take multiple behavioral forms. Overt choices are typically taken to represent a judgment, but more covert selection of one stimulus over another can emerge as approach to or avoidance of a stimulus. The evolutionary and cognitive theoretical frameworks of the study of affect converge on the notion that appraisal serves as an interface between input and output, input being sensory information and output being appropriate behavior. This appraisal is what enables interpretation and associability of stimuli for making decisions about them or otherwise fitting them into a rational framework.

Forming affective associations plays a great role in how people live their lives. Linking two sensory stimuli, where one stimulus has an inherent affective value, appetitive or aversive, gives grounds for salience of the second stimulus. A stimulus that used to be neutral or meaningless, through repeated pairing with an affectively laden stimulus, gradually acquires value and becomes important. This salience of a stimulus can serve to attract attention or to motivate behavior. A choice can be made based on this affective value, a choice further capable of guiding behavior. Thereby, goal directed behavior can arise from associative learning. These concepts: motivation, attention, decision making, and goal directed behavior influence our lives to a great degree. This is why affect is a cornerstone in guiding behavior.

Extensive research suggests that a brain structure deep in the temporal lobe, the amygdala, plays an important role in acquiring the key link between positive or aversive

affective value and a given stimulus (Everitt et al., 1989; Everitt et al., 1991; Hiroi & White, 1991; Davis, 1992: Gallagher and Chiba, 1996; Hatfield et al., 1996; LeDoux, 1996; Balleine et al., 1997; Killcross et al., 1997; Malkova et al., 1997; Whalen, 1998;). The amygdala is anatomically situated to serve as an interface between sensory input and learned behavioral output. The amygdala is comprised of a set of intercalated nuclei that receive direct sensory input and provide output to a variety of cortical and subcortical structures. The basolateral complex of the amygdala (BLA) receives most of the sensory input to this structure, while the central nucleus of the amygdala (CeA) receives most of its input from the BLA and provides the bulk of the output from the amygdala.

The first two chapters of this thesis are primarily focused on the role the basolateral nucleus of the amygdala plays in the affective appraisal of visual sensory cues. Animal models and novel behavioral tasks were designed to characterize this role. Incorporated in the behavioral design was the use of neutral visual stimuli paired with reinforcers that range from positive to negative. Ultimately, this served to encourage the formation of preferences for the visual cues paired with these reinforcers.

Numerous theoretical perspectives attempt to explain the way in which stimuli acquire value and guide behavior. Whereas many of these perspectives invoke associative learning as the basic mechanism of value acquisition, the subtleties of the theories give rise to controversy regarding what is being associated.

According to Lang (1995), affect in humans is composed of two key components: valence and arousal. Here, valence is described as a continuum, with different positive and negative sensory values corresponding to different points on an axis (see Figure 1). In order to survive, any given animal needs to respond with an appropriate behavior to a

given sensory stimulus. This response often requires that the sensory stimulus be evaluated based on its rewarding or aversive qualities. This evaluation in turn requires an association of the stimulus and a given reward value, i.e. “reward currency” (Rolls, 1999). It would not be evolutionarily adaptive to grossly overestimate or underestimate the reward value of a given sensory stimulus. Thus, from a natural selection perspective, it pays to recognize that a more evolutionarily fit organism is going to have a full grasp of this "reward currency" on a valence continuum (Rolls, 1999).

This kind of associative learning is known as "stimulus-affect" learning, since it involves a component of appraisal. Stimulus – affect learning is a subtype of more general stimulus – stimulus learning, which does not necessarily have an affective component, for example visuospatial associations, or discrimination of visual stimuli (Baxter and Murray, 2002). This type of learning is qualitatively different from the older "stimulus - response" learning, which is not based on cognitive appraisal, but on direct transfer of incentive value from the stimulus to the response (Everitt and Robbins , 1992, Furedy, 1992).

Appropriate behavioral output elicited in accordance with the emotional appraisal of sensory input has also been described as a type of goal oriented, motivated behavior (Derryberry and Tucker, 1994). Thus, importantly, affect is inextricably linked with decision making processes that include attending to stimuli, choice selection, and perceptual processing.

During the associative learning process, the accurate perception of the sensory qualities of a salient stimulus is just as important as the accurate perception of the incentive value of that stimulus (Everitt and Robbins, 1992; Balleine et al., 1997).

Sensory qualities such as auditory, visual, or tactile qualities are inherent properties of a stimulus. Such sensory qualities are associated with the motivational value of a reinforcer, such that the sensory qualities alone gain significance (Balleine, 2005).

Theoretically, the amygdala has been assigned numerous roles in affective learning and memory. Many theoretical views of the way in which a stimulus acquires value have been mapped directly onto the amygdala and its associated circuitry.

It has been shown that the amygdala is involved differentially in neural mechanisms underlying a) negative affect (Armony et al., 1995; Davis, 1997; LeDoux, 1992), b) positive affect (White and McDonald, 1993; Kesner and Williams, 1995; Salinas and McGaugh, 1995), c) memory enhancement based on physiological arousal (McGaugh et al., 1992; Cahill et al., 1995), and d) modulation of the cardiovascular response (Gallagher and Kapp, 1982; Buchanan and Powell, 1993; Powell et al., 1997), which is a direct correlate of physiological arousal, the second key component of affect according to Lang (1995). The valence dimension of affect, described by the degree to which a sensory stimulus is positive or aversive, may actually be tightly intertwined with the arousal component of affect, described by the degree of autonomic neural activation as well as the central nervous system mechanisms of arousal (Lang, 1995). This link of arousal and valence may not be dissociable, as suggested by Lang (1995), but may occur in concert, so that stronger preferences or aversions have both more valence and more arousal involved in their formation, while the formation of weaker preferences involves less of these dimensions (see Figure 1). There are a number of new experimental studies, on both humans and animals that support the existence of this arousal – valence interaction (Balleine, 2005; Winston et al., 2005).

While the valence dimension of affect contributes more to the sensory aspect of stimulus – stimulus associations, the arousal dimension of affect participates more in modulating these associations through central nervous system arousal mechanisms which involve stress factors, noradrenaline, and associated neural structures. The amygdala is involved in the appraisal of stressful information (Davis, 1992), and is directly involved in CNS arousal modulation (McGaugh et al., 1996, Roozendaal, 1999a; Roozendaal et al., 1999b; Ferry et al., 1999c, Miyashita and Williams, 2002; Curtis et al., 2002; Koob and Le Moal, 2001). Furthermore, amygdalar nuclei have been shown to participate directly in the modulation of heart rate and blood pressure (Gallagher and Kapp, 1982; Powell et al., Soltis et al., 1997, Sajdyk and Shekhar, 1997, Lewis et al., 1989). Thus, the amygdala is a prime structure for further investigation of its concurrent involvement in both the valence and arousal dimensions of affect. The explanation of how the amygdala differentially contributes to such a variety of processes may lie in its varied patterns of neuronal projections through which it connects to the rest of the brain and body (see Figure 2).

The functional significance of the central and basolateral amygdala nuclei includes a multitude of behavioral and autonomic regulatory mechanisms. The amygdala is involved in forming stimulus – stimulus (SS) associations when one of the stimuli is laden with affective value. This thesis provides support for the hypothesis that the basolateral amygdala has a dual function in associative learning, subserving regulatory mechanisms for both the valence and the arousal dimensions of affect and linking the two together to assign affective value to stimuli.

The basolateral amygdala receives sensory input from cortical sensory processing areas as well as from thalamic sensory nuclei (Amaral et al., 1996, Heimer et al., 1997, McDonald, 1998; McDonald and Mascagni, 1996). These inputs enable the amygdala to assess the valence dimension of affect. The amygdala, including the basolateral complex of the amygdala, also has access to information about the level of arousal through feedback circuits from the autonomic nuclei in the brainstem (Liang and Chiang, 1994; Miyashita and Williams, 2002). Processing of sensory and physiological information in the same anatomical locus, namely the BLA, stimulates the formation of associations and improves memory processing for arousing information (McGaugh et al., 1996).

The distinct associative learning paradigms which have been implemented and tested in this thesis influence both the behavioral and the physiological output of the animal. The physiological regulatory functions of the amygdala are potentially subserved by the extensive interconnectivity between the amygdalar subnuclei, the hypothalamus, and the autonomic nuclei in the brainstem (NTS, NA, DMV) that may serve to modulate physiological response (Buchanan et al., 1994; Ricardo and Koh, 1979; Price, 2003).

The regulation of learning and behavioral functions of the amygdala is potentially influenced by the projections back to sensory cortices, as well as by the interconnectivity with motivational striatal and executive frontal cortices (Vuilleumier et al., 2004; McDonald, 1998; Amaral et al., 1996; Heimer et al., 1997).

Both the basolateral and the central amygdala nuclei have important functions in stimulus – stimulus learning. The CeA is more involved in acquiring both appetitive and aversive classical conditioning, which is more closely related to stimulus-response (SR) learning. This might be due to the high degree of CeA interconnectedness with the

BNST, and the substantial involvement of this nucleus in the striatopallidal circuitry which subserves simple SR learning (Robbins and Everitt, 1992; Heimer, 1997). The BLA is more involved in acquiring both appetitive and aversive instrumental conditioning, which is more closely tied to the concept of goal oriented behavior and associating actions with outcomes (Gallagher and Chiba, 1996). These associations are important in subsequent cognitive appraisals of stimulus value, which are potentially subserved by amygdala – OFC interactions (Gallagher and Chiba, 1996; Schoenbaum et al., 1998, Schoenbaum et al., 1999).

2. Current Studies

The overarching hypothesis that is tested herein is that the encounter of affectively significant learning situations elicits fast adaptive responses regulated by subcortical associative learning loci. In this hypothesis, affective evaluation of information and feedback to the brain about physiological arousal play a central part in neural control of organismal functions.

We have completed a series of experiments that address the involvement of the BLA in the regulation of associative learning, and the influence of associative learning in cardiovascular regulation. Employing a behavioral task with both appetitive and aversive stimuli that has previously elicited differential activation in BLA neurons (Quinn et al., in preparation), we have assessed the effect of neurotoxic lesions on the formation of preferences for visual objects. We also examined how preferences for these objects affect both overt, goal directed choice behavior, as well as more covert, instrumental learning measures.

Additionally, we measured cardiovascular response in rats during baseline non-contingent appetitive behavior, and during a switch to an appetitive contingent context. The modulation of cardiovascular response by the BLA, through the use of transient BLA inactivation, was explored with respect to the potential role for this modulation on the interaction between physiological arousal and behavioral output.

The regulatory role that the BLA plays in associative learning and in the modulation of physiological arousal has important implications for the neural control of organismal adaptation to the environment. The discussion in the following chapters also focuses on the integration between this amygdalar function and what is known about the neural control of the adaptive process.

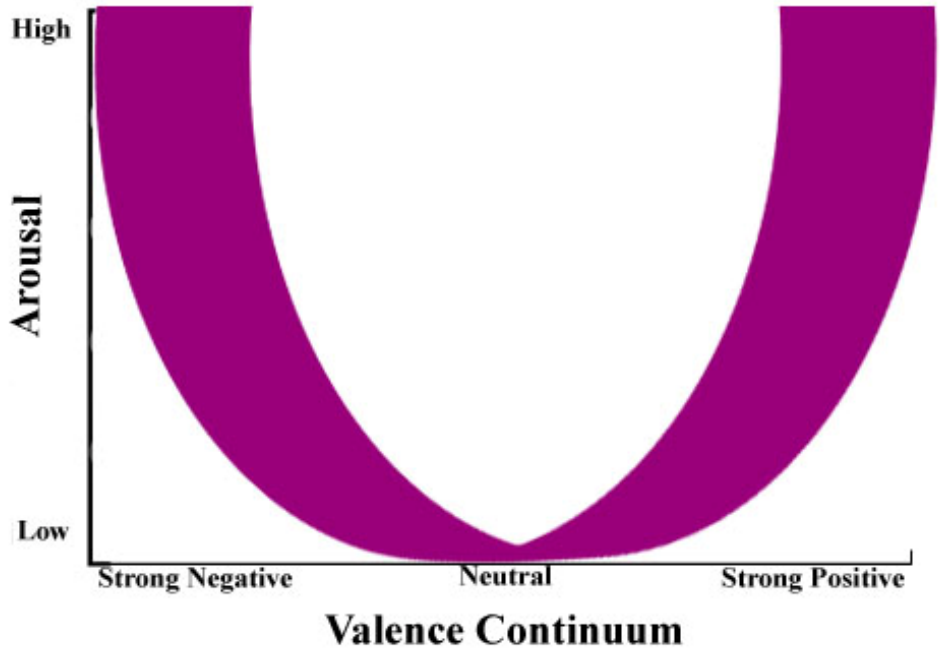


Figure 1.1 A schematic representation of the two dimensions comprising affect 1) the valence dimension, with different positive and negative values corresponding to different points on this continuum, also described by the degree to which a sensory stimulus is positive or aversive, and 2) the arousal dimension, described by the degree of activation of the central nervous system mechanisms of arousal. This link of arousal and valence may not be dissociable (Lang, 1995). *Schematically drawn by I.Y. Merzlyak*

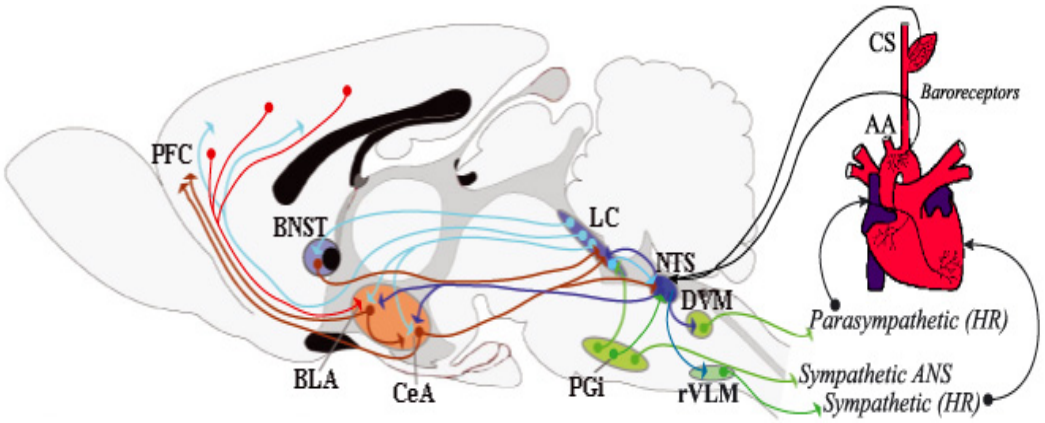


Figure 1.2 The circuitry underlying the cognitive, behavioral, and the physiological responses discussed in this thesis includes learning, motivational, and physiological neural connections This figure depicts the interconnectivity between the amygdala components (BLA and CeA) and the cognitive (PFC – Prefrontal cortex), motivational (BNST – Bed Nucleus of Stria Terminalis), and physiological (LC – locus coeruleus, NTS – nucleus of the solitary tract) loci that participate in the elicited physilogical and behavioral effects. There are numerous structures (some not shown) also associated with these loci that together coordinate and exert these effects. (Abbreviations: DVM – dorsal motor nucleus of the vagus, PGi – nucleus peri-gigantocellularis, rVLM – rostral ventro lateral medulla, AA – aortic arch, CS – carotid, ANS – autonomic nervous system). *Schematically drawn by I.Y. Merzlyak*

3. References

Alheid, G. F., & Heimer, L. (1988). New perspectives in basal forebrain organization of special relevance for neuropsychiatric disorders: the striatopallidal, amygdaloid, and corticopetal components of substantia innominata. Neuroscience, 27(1), 1-39.

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.

Arana FS, Parkinson JA, Hinton E, Holland AJ, Owen AM, Roberts AC. Dissociable contributions of the human amygdala and orbitofrontal cortex to incentive motivation and goal selection. J Neurosci. 2003 Oct 22;23(29):9632-8.

Armony, J. L., Servan-Schreiber, D., Cohen, J. D., & LeDoux, J. E. (1995). An anatomically constrained neural network model of fear conditioning. Behav Neurosci, 109(2), 246-257.

Balleine BW. Neural bases of food-seeking: Affect, arousal and reward in corticostriatolimbic circuits. Physiol Behav. 2005 Dec 15;86(5):717-30. Epub 2005 Oct 27

Baxter, M. G., & Murray, E. A. (2002). The amygdala and reward. Nat Rev Neurosci, 3(7), 563-573.

Baxter, M. G., Parker, A., Lindner, C. C., Izquierdo, A. D., & Murray, E. A. (2000). Control of response selection by reinforcer value requires interaction of amygdala and orbital prefrontal cortex. J Neurosci, 20(11), 4311-4319.

Curtis AL, Bello NT, Connolly KR, Valentino RJ. Corticotropin-releasing factor neurones of the central nucleus of the amygdala mediate locus coeruleus activation by cardiovascular stress. J Neuroendocrinol. 2002 Aug;14(8):667-82.

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. J Neuropsychiatry Clin Neurosci, 9(3), 382-402.

Davis, M., & Whalen, P. J. (2001). The amygdala: vigilance and emotion. Mol Psychiatry, 6(1), 13-34.

Derryberry, Douglas & Tucker, Don M. (1994). Motivating the focus of attention. In:

Everitt, B. J., Cador, M., & Robbins, T. W. (1989). Interactions between the amygdala and ventral striatum in stimulus-reward associations: studies using a second-order schedule of sexual reinforcement. Neuroscience, 30(1), 63-75.

Everitt, B. J., Morris, K. A., O'Brien, A., & Robbins, T. W. (1991). The basolateral amygdala-ventral striatal system and conditioned place preference: further evidence of limbic-striatal interactions underlying reward-related processes. Neuroscience, 42(1), 1-18.

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. Behav Neurosci, 108(1), 210-212.

Freese JL, Amaral DG., The organization of projections from the amygdala to visual cortical areas TE and V1 in the macaque monkey, J Comp Neurol. 2005 Jun 13;486(4):295-317.

Furedy, J. J. (1992). Reflections on human Pavlovian decelerative heart-rate conditioning with negative tilt as US: alternative approaches. Integr Physiol Behav Sci, 27(4), 347-355.

Gallagher, M., & Chiba, A. A. (1996). The amygdala and emotion. Curr Opin Neurobiol, 6(2), 221-227.

Gilbert PE, Kesner RP. 2002. The amygdala but not the hippocampus is involved in pattern separation based on reward value. Neurobiol Learn Mem May;77(3):338-53)

Gottfried JA, O'Doherty J, Dolan RJ. Encoding predictive reward value in human amygdala and orbitofrontal cortex. Science. 2003 Aug 22;301(5636):1104-7.

Hatfield, T., Han, J. S., Conley, M., Gallagher, M., & Holland, P. (1996). Neurotoxic lesions of basolateral, but not central, amygdala interfere with Pavlovian second-order conditioning and reinforcer devaluation effects. J Neurosci, 16(16), 5256-5265.

Heimer, L., Alheid, G. F., de Olmos, J. S., Groenewegen, H. J., Haber, S. N., Harlan, R. E., & Zahm, D. S. (1997). The accumbens: beyond the core-shell dichotomy. J Neuropsychiatry Clin Neurosci, 9(3), 354-381.

Hiroi, N., & White, N. M. (1991). The lateral nucleus of the amygdala mediates expression of the amphetamine-produced conditioned place preference. J Neurosci, 11(7), 2107-2116.

Kesner, R. P., & Williams, J. M. (1995). Memory for magnitude of reinforcement: dissociation between the amygdala and hippocampus. Neurobiol Learn Mem, 64(3), 237-244.

Koob, G.F. and Le Moal, M., 1997. Drug abuse: Hedonic homeostatic dysregulation. Science 278, pp. 52-58.

Lang, P. J. (1995). The emotion probe. Studies of motivation and attention. Am Psychol, 50(5), 372-385.

LeDoux JE, Sakaguchi A, Reis DJ. Strain differences in fear between spontaneously hypertensive and normotensive rats. Brain Res. 1983 Oct 24;277(1):137-43.

LeDoux, J. E. (1993). Emotional memory systems in the brain. Behav Brain Res, 58(1-2), 69-79.

LeDoux, J. E., Iwata, J., Pearl, D., & Reis, D. J. (1986). Disruption of auditory but not visual learning by destruction of intrinsic neurons in the rat medial geniculate body. Brain Res, 371(2), 395-399.

Liang, KC. And Chiang, TC. (1994) Locus coeruleus infusion of clonidine impaired retention and attenuated memory enhancing effects of epinephrine. Society for Neuroscience Abstracts, 20, 153.

Malkova, L., Gaffan, D., & Murray, E. A. (1997). Excitotoxic lesions of the amygdala fail to produce impairment in visual learning for auditory secondary reinforcement but interfere with reinforcer devaluation effects in rhesus monkeys. J Neurosci, 17(15), 6011-6020.

McDonald, A. J. (1998). Cortical pathways to the mammalian amygdala. Prog Neurobiol, 55(3), 257-332.

McDonald, A. J., & Mascagni, F. (1996). Cortico-cortical and cortico-amygdaloid projections of the rat occipital cortex: a Phaseolus vulgaris leucoagglutinin study. Neuroscience, 71(1), 37-54.

McDonald, R. J., & White, N. M. (1995). Information acquired by the hippocampus interferes with acquisition of the amygdala-based conditioned-cue preference in the rat. Hippocampus, 5(3), 189-197.

McEwen, BS. Allostasis and allostatic load: implications for neuropsychopharmacology Neuropsychopharmacology, Feb 2000(a), 22(2):108-24.

McGaugh, J. L., Cahill, L., & Roozendaal, B. (1996). Involvement of the amygdala in

memory storage: interaction with other brain systems. Proc Natl Acad Sci U S A, 93(24), 13508-13514.

McGaugh, J.L., Ferry B, Vazdarjanova, A, Roozendaal, B. (2000). Amygdala: role in modulation of memory storage. In The Amygdala. Edited by JP Aggleton. New York City: Oxford University Press, 391-412.

McGaugh, J.L., Introini-Collison ,I.B., Cahill, L., Kim, M., Liang, K.C.(1992). Involvement of the amygdala in neuromodulatory influences on memory storage. In The Amygdala. Edited by JP Aggleton. New York City: Wiley-Liss; (1992):431-452.

Miyashita, T., & Williams, C. L. (2002). Glutamatergic transmission in the nucleus of the solitary tract modulates memory through influences on amygdala noradrenergic systems. Behav Neurosci, 116(1), 13-21.

Pitkanen, A., Savander, V., & LeDoux, J. E. (1997). Organization of intra-amygdaloid circuitries in the rat: an emerging framework for understanding functions of the amygdala. Trends Neurosci, 20(11), 517-523.

Pitkanen, A., Stefanacci, L., Farb, C. R., Go, G. G., LeDoux, J. E., & Amaral, D. G. (1995). Intrinsic connections of the rat amygdaloid complex: projections originating in the lateral nucleus. J Comp Neurol, 356(2), 288-310.

Roberts, AJ; Heyser, CJ; Cole, M; Griffin, P; Koob, GF. Excessive ethanol drinking following a history of dependence: animal model of allostasis. Neuropsychopharmacology, 2000 Jun, 22(6):581-94.

Rolls, E.T., (1999). The Brain and Emotion. Oxford University Press, Oxford, England.

Roozendaal, B. (2000). 1999 Curt P. Richter award. Glucocorticoids and the regulation of memory consolidation. Psychoneuroendocrinology, 25(3), 213-238.

Roozendaal, B., Williams, C. L., & McGaugh, J. L. (1999). Glucocorticoid receptor activation in the rat nucleus of the solitary tract facilitates memory consolidation: involvement of the basolateral amygdala. Eur J Neurosci, 11(4), 1317-1323.

Salinas, J. A., & McGaugh, J. L. (1995). Muscimol induces retrograde amnesia for changes in reward magnitude. Neurobiol Learn Mem, 63(3), 277-285.

Schoenbaum G, Chiba AA, Gallagher M. Orbitofrontal cortex and basolateral amygdala encode expected outcomes during learning. Nat Neurosci. 1998 Jun;1(2):155-9.

Schoenbaum G, Setlow B, Saddoris MP, Gallagher M. Encoding predicted outcome and acquired value in orbitofrontal cortex during cue sampling depends upon input from basolateral amygdala. Neuron. 2003 Aug 28;39(5):855-67.

Schoenbaum, G., Chiba, A. A., & Gallagher, M. (1999). Neural encoding in orbitofrontal cortex and basolateral amygdala during olfactory discrimination learning. J Neurosci, 19(5), 1876-1884.

Sterling, Peter; Eyer, Joseph Allostasis: A new paradigm to explain arousal pathology. In: Shirley Fisher, Ed; James Reason, Ed; et al. Handbook of life stress, cognition and health. John Wiley & Sons: Chichester, England UK, 1988. p. 629-649 of xxxiii, 750pp.

Swanson, L. W., & Petrovich, G. D. (1998). What is the amygdala? Trends Neurosci, 21(8), 323-331.

Tanaka JW, Kiefer M, Bukach CM. A holistic account of the own-race effect in face recognition: evidence from a cross-cultural study. Cognition. 2004 Aug;93(1):B1-9

Taylor, B. K., Holloway, D., & Printz, M. P. (1994). A unique central cholinergic deficit in the spontaneously hypertensive rat: physostigmine reveals a bradycardia associated with sensory stimulation. J Pharmacol Exp Ther, 268(3), 1081-1090.

Vuilleumier P, Richardson MP, Armony JL, Driver J, Dolan RJ, Distant influences of amygdala lesion on visual cortical activation during emotional face processing, Nat Neurosci. 2004 Nov;7(11):1271-8. Epub 2004 Oct 24.

White NM, McDonald RJ. Acquisition of a spatial conditioned place preference is impaired by amygdala lesions and improved by fornix lesions. Behav Brain Res. 1993 Jun 30;55(2):269-81

White, N. M. (1989). Reward or reinforcement: what's the difference? Neurosci Biobehav Rev, 13(2-3), 181-186.

Winston JS, Gottfried JA, Kilner JM, Dolan RJ. Integrated neural representations of odor intensity and affective valence in human amygdala. J Neurosci. 2005 Sep 28;25(39):8903-7.

II

Basolateral Amygdala in choice behavior

1. Abstract

The basolateral amygdala complex (BLA) has a well established and important role in learning the associations between neutral stimuli and rewards or punishments. This chapter is primarily focused on explicating the role the BLA plays in the affective appraisal of visual sensory cues. Basolateral amygdala sends upstream bidirectional projections to sensory cortical areas and to the prefrontal cortex, underscoring its influence on learning and decision making. Basolateral amygdala directs a large portion of its output through the central nucleus to the brainstem motor effector nuclei, underscoring its control over behavioral outcomes. The current experiment tested the effects of neurotoxic BLA lesions during an associative learning task in which neutral visual objects were paired with either positive or aversive food pellets. It was demonstrated that the BLA contributes to the elicitation of overt choice behavior when picking between pairs of affectively significant visual objects. It was found that the BLA plays an important role in early learning of the distance relationships on a spectrum of affective values associated with objects.

2. Introduction

The amygdala is conveniently located with respect to neural connectivity, to serve as an interface between sensory input and learned behavioral output. Furthermore, it is suggested here that the amygdala is a processing hub of both sensory and affective

information. The current study examined the role of the amygdala in learning multiple visual stimuli of varied affective significance. This approach allows for the integration and dissociation of multiple theoretical perspectives regarding the functional role of the amygdala.

2.1 Anatomical Connectivity

The amygdaloid complex and the closely associated family of nuclei called the extended sublenticular amygdala are categorized by their afferent and efferent projections. The nuclei in the basolateral complex, as well as associated extended structures, receive most of the extra-amygdalar afferents. The central and medial nuclei, in addition to some extra-amygdalar afferent projections, receive most of their input as intrinsic connections from the basolateral complex and serve as the origin of most, but not all efferent projections from the amygdala. There are other amygdalar sub-nuclei that do not fit into these divisions, among them the cortical nucleus which has the distinction of receiving input directly from the main olfactory bulb as well as the primary olfactory cortex (McDonald, 1998).

The basolateral complex (BLA) includes the lateral, basolateral, basal, and basomedial nuclei, and is made up of excitatory pyramidal neurons and inhibitory GABA mediated interneurons. This cellular composition is reminiscent of the cortex, which is evidenced by the specification of this part of the amygdala as an “archicortical” structure from both evolutionary and developmental perspectives (Swanson and Petrovich, 1998; Heimer et al., 1997). The maturation of BLA in rats is complete by postnatal day 14 as judged by the number of cells, cell density, and the number of subdivisions of BLA, all

measured with morphometrical methods (Berdel et al, 1997). In adult rats, cats, and monkeys the BLA projects to a variety of primary sensory cortices, including occipital area 1, A1, and primary gustatory cortex (Price et al., 1987). The BLA also receives direct projections from occipital area 2, insular cortex, and many other higher order associative sensory areas, including area Te2 in a rat, anterior cingulate, piriform, and auditory cortices. (Amaral et al., 1996, Heimer et al., 1997, McDonald, 1998; McDonald and Mascagni, 1996) Thus, the BLA receives both unimodal and putative polymodal input from sensory cortices. An alternative route by which BLA receives sensory information is from the thalamic nuclei. It also projects bidirectionally to the ventral striatum, and unidirectionally to both shell and core of the nucleus accumbens, as well as dorsal striatum (Heimer et al., 1997; Swanson and Petrovich, 1998).

Both the basolateral complex and the central nucleus also receive direct noradrenergic projections from the locus coeruleus (LC) and from the nucleus of the solitary tract (NTS) (Fallon and Ciofi, 1992; Liang and Chiang, 1994; Ricardo and Koh, 1978; Valentino and Aston-Jones, 1995). The CeA also sends projections to both NTS and LC (Van Bockstaele et al., 1998; Koob, 1999). Projections from LC play a major role in affective memory modulation in BLA (Liang and Chiang, 1994). The projections from NTS may play a role in autonomic cardiovascular feedback in addition to their established effect on memory modulation (Roozendaal et al., 1999; Williams and McGaugh, 1993, Miyashita and Williams, 2002). There is a functionally significant bidirectional projection from the BLA to the orbitofrontal part of the prefrontal cortex (OFC or OFC equivalent) in rats, cats, and monkeys (Price et al., 1996, Amaral et al., 1997, McDonald, 1998).

There is a very high degree of interconnectivity within the basolateral complex, with the flow of information generally coursing from the lateral to the basal and accessory basal sub-nuclei. There are multiple pathways interconnecting these sub-nuclei, and all of them project separately to the central and medial nuclei of the amygdala (Pitkanen and Amaral, 1991; Stefanacci et al., 1992).

The central and medial amygdalar nuclei (CeA and MeA) send projections to the hypothalamus, and the central nucleus sends downstream projections to the autonomic and somatosensory areas of the brainstem where it can elicit a sympathetic nervous system response. The conventional borders of CeA and MeA extend neurohistochemically into the sublenticular substantia inominata (SI) and to the bed nucleus of the stria terminalis (BNST) (Heimer et a., 1997). It is through these connections that the CeA has access to the entire cortical mantle. The interconnections between CeA, MeA, bed nucleus of the stria terminalis, and the sublenticular areas are so prominent that the sublenticular and bed nucleus of stria terminalis regions are often referred to as "extended" or "sublenticular" amygdala (Heimer et al., 1997). The connectivity pattern of the sublenticular amygdala is similar to that of the CeA and MeA. Through the direct brainstem and sublenticulo-cortical connections, the CeA is able to modulate neurochemical systems in much of the brain.

Amygdalar subdivisions have also been viewed from the perspective of functional and structural divisions of the rest of the brain (Swanson and Petrovich, 1998). Under the structural criteria, the BLA is a cortical structure, while CeA and MeA are striatal structures, which may be relevant to their degree of involvement in stimulus – stimulus versus stimulus – response learning strategies. Under the functional criteria, the

basolateral complex is part of the frontotemporal system, except the basomedial nucleus which is part of the olfactory system (Swanson and Petrovich, 1998). The anatomical information reviewed serves to inform us that the amygdala is optimally situated to serve a proposed role in acquiring stimulus-stimulus associations, in addition to modulating physiological states.

2.2 Role of the amygdala in aversive associative learning

There is a large body of work mapping learning of stimulus – stimulus association onto the amygdala and associated circuitry. The majority of this work has focused on the neural circuitry underlying fear associations. It has been well established that the described connectivity of the amygdala plays a significant role in one aspect of associative learning involving affective appraisal of a stimulus, fear. (Davis, 1992: LeDoux, 1992 ; Kim & Fanselow, 1992; Davis and Whalen, 2002) Fear conditioning, as one form of aversive conditioning, has been broadly applied to investigate the formation of a fear response to a stimulus. The functional significance of this is that an aversive stimulus, such as electrical footshock, becomes associated with a sensory cue, such as an auditory tone or a visual light flash through repeated simultaneous exposure to both. As a result of this "training" the rat reacts to the tone or light alone in a similar way as it reacts to the footshock alone. This response is called "startle response" and it involves freezing, jumping up in the air, and exhibiting an appropriate autonomic response. The circuitry supporting this form of conditioning has been well laid out: the lateral nucleus receives sensory input from the thalamus and from the cortex, while the basal nuclei receive sensory input only from the cortex. These nuclei are highly interconnected. The

basolateral complex relays the information to the central nucleus which in turn sends projections to the brainstem. Animals with bilateral amygdala lesions (either BLA or CeA) do not learn to associate a sensory cue with footshock. (LeDoux, 1990) The aversive stimuli used in fear conditioning are always extreme in valence and elicit physiological arousal.

Research on the fear circuitry has established that direct thalamo-amygdalar connections are as important for fear conditioning as the indirect thalamo-cortico-amygdalar connections. Fear conditioning is only completely abolished when the entire auditory thalamus is removed (Romanski and LeDoux, 1992). The amygdala receives other types of sensory input through the thalamus as well, including somatosensory and visual information. Although auditory fear conditioning is thalamus dependent, visual fear conditioning persists following elimination of visual thalamic input (LeDoux et al., 1986). This emphasizes the significance of direct projections to the amygdala from the visual cortex, and from the associative sensory cortices. This also underscores the importance of using various sensory cues in associative learning paradigms since the neural circuitries could be sense specific with respect to the amygdala.

The literature discussed above established the notion that the BLA is responsible for all types of associative fear learning. However, an elegant within subject study looked at the influence of selective BLA or CeA lesions on both classical and operant fear conditioning simultaneously (Killcross et al., 1997). This study replicated the findings of the traditional fear conditioning experiments; it showed the necessity of BLA for classically conditioned fear. The implementation of an alternative paradigm within an operant conditioning framework revealed that the extent to which fear conditioning relies

on the integrity of BLA is dependent on whether or not the rat is allowed to exert influence over the delivery of the aversive stimulus. The more influence the rat could exert, the more BLA lesion interfered with performance (Killcross et al., 1997). Rats with CeA lesions demonstrated the opposite effect, so that a double dissociation was found between the effects of BLA and CeA lesions. These findings demonstrate that the neural circuitry of fear conditioning is more complex than a serial input-output pathway described above. The BLA projections to the pre-frontal cortex and the striatum may make the BLA a part of an alternative affective associative neural pathway which is directly involved in goal oriented behavior. Specifically, making a choice to avoid a punisher and then executing appropriate avoidance behavior is an important mechanism, with stimulus –affect association at its heart. The series of experiments proposed here suggests that BLA is critical to the neural pathways underlying this "cognitive" mechanism.

2.3 Role of the amygdala in appetitive associative learning

Although the majority of current research implicates the amygdala in fear associative learning, it also plays a substantial role in mediating positive affective appraisal of sensory stimuli. The amygdala is an integral part of the neural circuitry underlying numerous appetitive associative learning tasks (Kesner et al., 1989; Cador et al., 1989; Everitt and Robbins, 1992; Hamann et al., 1999; Parkinson et al., 2000). There has been a wealth of research in recent years examining the selective involvement of BLA and CeA in positive affective conditioning paradigms (Holland et al., 2001, Balleine et al., 2003, Blundell et al., 2001, Schoenbaum et al., 1998, Chiba et al., 2002).

The literature on this subject seems to agree that in parallel to aversive conditioning, in appetitive conditioning the BLA bears more influence in instrumental conditioning paradigms, while the CeA seems more involved in Pavlovian responses (Everitt et al., 2000). Furthermore, there are some aspects of appetitive conditioning that do not seem to depend on amygdalar processing, such as simple food cup approach, where rats learn to approach a food receptacle after food delivery (Everitt and Robbins, 1992). The striatal and ventral pallidum circuitry is hypothetically involved in this latter type of learning, described as stimulus-response (SR) learning (Everitt and Robbins, 1992, Alheid and Heimer, 1988, Parkinson et al., 2000).

The basolateral complex of the amygdala has been implicated in processing information about the current reward value of a sensory stimulus following a reward devaluation process (Hatfield et al., 1996). In a selective reward devaluation paradigm, rats with BLA lesions learned that two different instrumental responses predicted two equally tasty foods with different sensory qualities (solid and liquid of equal sweetness) (Blundell et al., 2001). However, BLA lesioned animals could not learn to discriminate between these taste outcomes after one of them was devalued (Blundell et al., 2001). The central nucleus of the amygdala, unlike BLA, is more important for increased orienting to the stimulus (CS) that becomes associated with rewarding outcome (Holland et al., 2001). It is still somewhat controversial whether the amygdala is important in linking specific sensory qualities of the stimuli or the motivational value of the stimuli (Blundell et al., 2001; Baxter and Murray, 2002; Everitt and Robbins, 1992; Everitt et al., 2000). A more likely outcome is involvement in both, suggested by the amygdalar interconnectivity with

CNS arousal mechanisms (Williams and McGaugh, 1993; Van Bockstaele et al., 1998) alongside its feedback to the sensory cortices (McDonald, 1998).

2.3.1 Involvement of amygdala in memory for reward magnitude

The research described below focuses specifically on the role of the amygdala in differentiating magnitude of reward in associative learning and memory paradigms, one aspect of amygdala research that has not gained much attention in the recent years.

One appetitive learning experiment demonstrated that the amygdala is necessary for accessing information about previously formed relationships between affectively laden stimuli (food at the next trial) and different reward magnitudes. This study involved forming associations between food stimuli of different reward magnitude (high versus low sugar) and the availability of food on the following test trial. (Kesner and Williams, 1995) The rats learned that the high sugar food was always followed by reward at the next trial, while the low sugar food was never followed by reward at the next trial. The time it took the rats to look for the reward at the next trial was used as a measure of whether they learned this task. After the rats mastered this task, they underwent selective amygdala lesions. The results showed that amygdala lesioned rats were unable to remember postoperatively which food was followed by reward.

It is important to note that both stimuli in this experiment were affectively laden, and there was no neutral stimulus in this paradigm. The lesioned rats still had the same food preferences as the normal rats, however, the lesioned rats could not act to their advantage based on the difference in reward value of the stimuli (Kesner and Williams,

1995). This indicates that the amygdala is involved in differentiating the reward values of stimuli after the values are learned, i.e. at the performance stage.

Using Conditioned Place Preference (CPP) appetitive paradigms, it has been shown that the amygdala is necessary for remembering a location preference based on a magnitude of reward delivered at that location (White and Packard, 1991; White and McDonald, 1993; Fuchs et al., 2001). While exploring an eight arm maze, rats demonstrate a preference for those arms of a maze where they find more food relative to the other arms (White and Packard, 1991; White and McDonald, 1993). During the test stage the food was no longer present; the rats got to freely explore the maze, and the time spent in each arm was recorded. Rats prefer the places previously associated with high reward (more food). Rats with amygdala lesions no longer differentiate between the places previously associated with high reward versus the previously low reward locations.

A related study demonstrated that rats with BNST lesions are impaired at learning the differences in costs of a reward (Brown et al., 1996). Rats learned to press a bar to get a food reward, but with each trial the number of bar presses necessary to obtain the same reward increased. Normal rats will press the bar with more pauses between presses as the number of necessary presses increases. Rats with bilateral lesions of sublenticular extended amygdala (SEA or BNST), which is highly interconnected with CeA, will press the lever with the same urgency whether they need to press it once to obtain a food pellet or if they need to press it 16 times to obtain the same pellet. The rats still remember that bar pressing is associated with food reward, but they no longer can differentiate between the relative difficulties of obtaining this reward. This experiment has components of both

stimulus-stimulus and stimulus-response learning, and while SR is preserved (bar pressing for a reward), SS seems to be impaired. If the cost of a reward is indirectly correlated with affective valence, then the BNST lesioned rats cannot tell the difference between different valence distances on the affect scale, an incentive value deficit.

2.3.2 Involvement of amygdala in the learning of appetitive associations

Several experiments show how amygdala nuclei influence the formation of affect – stimulus associations. For example, rats with BLA lesions are impaired at acquiring preferences for a location in a cocaine reinforced CPP task (Fuchs et al., 2002). In an appetitive instrumental task, when rats with excitotoxic BLA lesions are taught to perform different actions for foods that differ only in sensory qualities (liquid versus solid with same degree of sweetness), they are not impaired in acquiring this task (Blundell et al., 2001). However, if one of the appetitive stimuli is devalued by selective satiation, the performance of the operant actions is not adjusted selectively in lesioned as opposed to sham operated rats (Blundell et al., 2001). This provides further support for the BLA's role in associating appetitive stimuli with specific instrumental responses. On the other hand this study again brings up the importance of SR learning in appetitive paradigms. It is possible that the rats are able to correctly learn the initial responses to get the rewards because the striatopallidal pathways that largely mediate SR learning are intact.

A series of experiments involving the differential cost of reward was run by Salinas and McGaugh. Rats learned to expect a reward of a given magnitude (10 sugar pellets) at the end of a walkway, which made them reach the end of the walkway with a

certain latency. Following learning, if the amount of reward was reduced the rats would take longer to reach the food dish. Full amygdala lesions abolished the memory for reward change. An alternative interpretation of this phenomenon is that processing in the amygdala was necessary for learning the association between the new magnitude of reward and reaching the end of the walkway. While the animals had learned to exert a certain amount of effort in reaching this large reward, lesions of the amygdala prevented them from updating the value of reaching the end of the walkway, so the degree of effort stayed the same even while the magnitude of reward changed. This effect was also influenced by GABAa agonists and antagonists infused into the amygdala. Muscimol, a GABAa agonist, infused into the amygdala interfered with affective memory, while bicuculline, a GABAa receptor blocker, enhanced the memory for change in reward magnitude. Propranolol, a beta adrenergic antagonist, also interfered with affective memory enhancement for reward change in the same paradigm when infused into the amygdala. These experiments demonstrate clear involvement of several neurochemical systems in processing rewarding associations, or memory for reward magnitude, in the amygdala.

In sum, the amygdala is necessary for both acquisition and performance (learning and memory) stages of affect dependent associative learning. Also, both the amygdala and the extended amygdala are necessary for differentiations of reward values in associative learning. Controversy remains regarding the specific involvement of BLA and CeA in this appetitive circuitry, in part due to the lack of variety in sensory cues used in appetitive associative learning, as well as the specificity of the lesion techniques employed.

2.3.3 Electrophysiological recordings in the amygdala during associative learning tasks

To compliment the lesion studies, electrophysiological recordings of amygdala cells in freely behaving animals have been performed (Ono et al., 1995; Schoenbaum et al., 1998, Schoenbaum et al., 1999; Chiba et al., 2002). The firing data shows that neurons in the BLA quickly acquire selective firing properties to stimuli associated with either positive or aversive outcomes (Schoenbaum et al., 1999; Chiba et al., 2002).

One task involved using different odors to predict the availability of either a positive (sucrose solution) or a negative (quinine solution) consequence. The authors found that cells in amygdala begin to selectively fire in anticipation of either rewarding or aversive stimuli early in the learning process, before the rat overtly learns the correct behavior. Specifically, neurons in the BLA fired selectively in anticipation of these events after detecting the distinct olfactory stimuli. Further, the timing of these predictive firings preceded a similar firing pattern in the orbitofrontal cortex (Schoenbaum et al., 1999).

Another study that recorded single unit firing data in the basolateral amygdala of awake behaving rats used a task almost identical to the one used here (Chiba et al., 2002). Rats had to learn the motivational significance of Lego objects paired with rewarding and aversive food stimuli. This learning process was examined across six days of training and testing. Approaching the object in the pair of two objects that was associated with a tastier food pellet demonstrated successful acquisition of the motivational significance of the objects. During this task, a proportion of single units in the BLA fired selectively to

different objects based on the associated outcome of each object. These findings further implicate a role for the amygdala in encoding the associative significance of cues and highlight the use of valence information in the selection of a behavioral response (Chiba et al., 2002). This evidence is strongly supportive of BLA being a learning hub in the brain for stimulus – stimulus associations that have a component of affective appraisal.

2.4 The Current Investigation

In the current project we implemented a novel behavioral task that enabled us to examine the valence dimension of affect at discrete points on a continuum from positive to negative. This task involved looking at stimulus - stimulus associations between neutral objects and food stimuli of gradated valence (from positive to aversive) that result in formation of an affective preference scale for the objects. This task also had elements of stimulus – response learning which needed to be teased apart from the stimulus – affect learning during behavioral analysis. This experiment is without a precedent for several reasons: 1) we are looking at the associations between a series of visual/tactile stimuli and both positive and aversive reinforcers, 2) we are investigating the effect of selective BLA lesions on the learning of preferences for differential magnitude of reward, and 3) we are testing the involvement of the BLA in the acquisition, not performance, of affective associations.

To assess the necessity of the basolateral amygdala in establishing positive and negative object valence associations within the same learning paradigm, the experimental group of rats underwent bilateral basolateral amygdala lesioning prior to the acquisition

stage of a preference formation task wherein neutral objects were paired with outcomes of differing valence.

In order to investigate the role of the BLA in discrimination between stimuli that are close on the valence scale versus stimuli that are far apart on the valence scale, we used several levels of behavioral analysis to examine how behavioral and learning patterns differ between lesioned and non-lesioned rats.

3. Materials and methods

3.1 Subjects

All procedures and animal care adhered strictly to Society for Neuroscience, and institutional IACUC guidelines for experimental animal health, safety, and comfort. Subjects were 19 adult (4 month old at the beginning of experiment) hooded male rats (Long Evans), housed individually and weighing 350-450g at the beginning of the experiment. They were fed *ad libitum* until three weeks prior to training, then brought down to and maintained at 93% free feed weight, with water and food intake monitored and recorded daily. Water was available *ad libitum* throughout the experimental procedures. Rats were maintained on a 12 hour/12 hour light dark cycle. Rats were obtained from Charles Rivers Laboratories (Portage, ME). 9 animals were included in the experimental group and had BLA lesions, and 10 animals underwent vehicle infusions and served as controls.

3.2 Surgical Procedures: BLA lesions The experimental group had the BLA removed bilaterally, while the control group underwent the same surgeries but with

vehicle (acsf) infusions. Neurotoxic lesions of the basolateral amygdala complex were made under antiseptic conditions using N-methyl-D-aspartate (NMDA). Each rat was anesthetized with 2% isoflurane in an O_2 carrier. Each rat was then placed in a stereotaxic apparatus. An incision was made along the midline of the rat's head, and the underlying periosteal fascia was scraped to the side. Two holes were drilled through the skull, and a small slit was made in the underlying dura at each location to assist needle penetration. A Hamilton syringe (10 µl) with a 28 gauge permanent needle was used to make four injections of NMDA (diluted to 12 µg/µl in a 0.01M Phosphate Buffer solution, pH 7.4) Neurotoxic injections, or equal volume injections of vehicle (0.01M PBS alone), were made at stereotactic coordinates -2.8 mm posterior to bregma, 5.0 mm lateral from the midline (on either side), and ventral from the skull surface at -8.1 mm and -8.4 mm, respectively. Following the completion of all injections, the skull was cleaned, the wound sutured, and a topical antibiotic was applied to the exterior of the wound site. Upon completion of surgery, the rat was given intramuscular injections of Penicillin-G (30,000 units, Sigma, St. Louis, MO) and buprenorphine (0.05 mg/kg). Each rat was placed on a warm heating pad in a recovery cage and monitored during recovery from anesthesia. Following the surgeries, the rats were allowed 7 days of post-operative recovery before the initiation of behavioral training.

3.3 Behavioral Apparatus

An open field circular wood platform equipped with 177 food wells was used for testing rats on all tasks. This platform consists of three round layers of wood, the bottom one is solid, while the two top ones have 177 geometrically positioned round holes that

are 1 inch in diameter and go all the way through the boards. The two top boards are separated by wire netting which makes it impossible to reach the holes in the middle wood layer when the whole platform is assembled. The edible pellets (Noyes/Research Diets) used as stimuli in the experiment are placed in the holes of the top layer. All four kinds of pellets used in the experiments were placed in each food well under the netting (middle wood platform layer) to eliminate olfactory cueing. This open field apparatus, also called the "cheeseboard" was used for all of the experiments described here.

3.4 Behavioral Training3.4.1 Taste discrimination and consumption test

Initial training involved taste discrimination consumption tests for the rats. A basic taste discrimination control experiment was performed in order to ensure that both groups of rats (BLA lesioned and controls) were able to differentiate one taste from another and to demonstrate normal flavor preferences. In accordance with our pilot work, the custom Noyes pellets (PJ Noyes Inc./Research Diets, NH) were ordered and manufactured at concentrations such that rats responded most preferentially to pellets with the highest sucrose concentration (100%), relative to pellets with the low sucrose concentration (25%), low Quinine HCl concentration (0.002%), and least to pellets with the highest Quinine HCl concentration (0.02%). All pellets were manufactured with cellulose base. Sweet pellets with the 100% and 25% sucrose concentrations had no Quinine HCl, and bitter pellets with 0.002% and 0.02% Quinine HCl had no sucrose in their composition. In accordance with previous research on taste reactions among species, the rats were observed for universal hedonistic ("yum") and aversive ("yuck") reactions to the pellets (Berridge, 2000).

The rats were given consumption tests to determine their preferences for different kinds of food reinforcements. The same pellets as described above were used in this experiment. In order to test the ability of each rat to discriminate between the pellets, each rat had free access to two piles (equal in quantity) of the pellets. After 60 seconds the amount remaining in each pile was measured and compared. The piles of pellets were counterbalanced for side of presentation (Left v. Right) and presented on an exhaustive pairwise schedule.

Given that BLA lesioned rats have the same food preference patterns as controls, as well as appropriate behavioral reactions, it is assumed that any deficits observed in the main behavioral task are not due to the rats' inability to discriminate between rewards or alterations in basic taste preferences.

3.4.2 The affective preference formation, 4 object task During this task, rats are trained to associate a Lego object with a particular food pellet. An object set consists of four different Lego objects, one for each kind of pellet. The rats are trained with a given object set for six days, then given a break followed by a switch to a new object set.

This preference formation task consists of two kinds of trials: training, or acquisition, and test, or performance. The test and training trials are interspersed throughout the task. There are 10 training trials total, 5 before and 5 after the first test trial, and 6 test trials total. The training trials involve the exposure of the rat to all four Lego objects involved in the experiment, one at a time. The object is placed on the board five holes away from the door of the start box, with the suitable edible pellet underneath, then the rat is made to orient its nose to the door by the experimenter lightly tapping the

door from the outside. The start box is opened, and the rat has to run up to the object and push it over in order to reach the edible pellet. The rat spends time examining the object and eating or examining the pellet. As soon as the rat turns away from the object, the rat is returned to the start box, or alternatively, returns to the start box on its own volition. Then the object is taken off the board, and the next object out of the four is placed on the board instead, with the appropriate edible pellet underneath. The four objects are presented to the rat in random order, for five training trials total, so that the rat sees each object five times before the commencement of the test trials.

Each test trial is immediately preceded by a training trial which serves a dual purpose. First, the rat is given an opportunity to see all of the four objects immediately before a test trial, which serves to eliminate the need to keep the objects in memory. Second, each training trial that precedes a test trial is constructed in a way that places the two objects involved in that test in the middle of the training presentation. For example, if the test trial is Object 2 versus Object 3, the order of the object presentation in the training trial would be 1,2,3,4 or 4,2,3,1, but not 2,1,3,4 or 1,3,4,2. This feature of the experiment is designed to minimize the proactive and retroactive interference that might play a role in selecting the objects during a test trial.

Each test trial involves a presentation of two of the four objects to the rat at the same time. Both objects in the test trial, picked according to a set schedule, are placed on the board 12 holes away from the start box door, equidistant from the center of the start box and covering adjacent holes where the appropriate food pellets are placed (See Figure 3). The rat is oriented to the door of the start box in the same manner as in a training trial, and a test trial starts out exactly the same as a training trial. The rat had to

approach the two objects and pick one of the two to push over and examine or eat the pellet underneath. The rat was consistently prevented from pushing over the other object during the initial training for the experiment, and by the onset of data collection the rats generally do not try to push over both objects. As soon as the rat turns away from the object that was pushed, it is returned to the start box, or the rat returns to the start box on its own volition.

The rats completed training on at least six, and at most eight different objects sets.

3.4.3 Fear conditioning

At the conclusion of the behavioral testing on the preference task, the rats underwent training and testing on a delayed tone fear conditioning paradigm, adapted from Davis, 1992. Briefly, the animals were put in a behavioral chamber, with the house light on, and subjected to a 10 second tone, paired with a brief (1 sec) electrical shock, for 10 separate trials, within a single 12 minute training session. On the following day, the animals were put in a contextually different behavioral chamber, and subjected to a testing session, where the tone alone was presented for 10 seconds, in 10 separate trials, in a single 12 minute test session. The activity of the animals was measured throughout with an infrared sensor and the resulting activity data was output to a computer using the Graphics State software. The results of the fear conditioning experiment were used to verify the efficacy of the BLA lesions, and used to determine the inclusion criteria for the lesioned and the vehicle infused animals in the preference task behavioral analysis.

3.5 Histological methods

At the conclusion of behavioral training, each rat was euthanized using Nembutal (120 mg/kg, i.p. sodium pentobarbital). Following lethal injection, rats were perfused transcardially with 0.9% saline followed by 4% paraformaldehyde fixative. Brains were then removed and stored in the same 4% paraformaldehyde fixative solution used for perfusion. Three days before the brains were further processed, they were transferred to a 20% sucrose in phosphate buffer solution. Then the brains were frozen with dry ice, sectioned coronally (40 μm) using a sliding microtome, and every section was mounted onto gelatinized glass slides, and Nissl-stained. To ensure that a majority of fibers of passage are left intact, alternate sections were stained using a silver stain for visualizing fibers. The extent of lesions was determined by visual inspection of the slides under the microscope. The lesions were characterized according to size and location and used to determine which animals would be included in statistical analysis.

3.6 Statistical Analysis

The behavioral data from the open field object preference formation experiment were analyzed using JMP 4.0.2 (SAS institute, Cary, NC). For the "choice" data (which object out of a pair the rat picks), a three way repeated measures ANOVA was performed – two within variables (time blocks of training (4) and choices(6)), one between variable (lesion/vehicle group). Once the main effects became apparent, the choices were grouped into categories according to either valence (within versus between) or nearness of objects on the valence continuum (adjacent versus separate). Then two three way repeated measures ANOVA were performed on the different groupings of choices – two within variables (time blocks of training (4) and valence(2) or proximity (2)), one between

variable (lesion/vehicle group). Subsequently, step down ANOVAs and post-hoc comparisons were performed on choice data variables with significant main effects.

Microchoice and latency data analyses will be discussed in detail in Chapter 2.

4. Results

4.1 Histological results

Upon histological analysis of the infusion sites, the extent of the BLA lesions was quantified and rated according to the specific amgydalar subnuclei site of damage. A representative extent of the NMDA lesion site, as well as a representative vehicle infused BLA section, are shown in Figure 1A. At the completion of the experiment, after histological analysis of the brain sections, 4 out of the 19 rats were eliminated from the analysis based on either insufficient or too much incidental BLA damage (all 20% unilateral or bilateral BLA lesioned subjects were not included in the final analysis). Another 3 rats were eliminated based on erroneous lesion targeting. One more vehicle infused rat was eliminated based on aberrant endogenous gliosis in the BLA. At the conclusion of the analyses, there were 6 animals included in the control group and 5 animals included in the experimental group.

4.2 Behavioral results

4.2.1 Fear Conditioning

A one way ANOVA, with the lesion/vehicle group as the between variable, was performed on the rate of activity during the aversively conditioned tone. Rats with the

BLA lesions were significantly less likely to fear condition successfully than the vehicle infused animals (as shown by the main effect of group: $F=(9,51)$, $p=0.018$; see Figure 2).

4.2.2 Preference Formation Task

In the behavioral analysis for this experiment it was found that the rats with intact basolateral amygdalae learned to choose objects according to preference significantly better than the rats with lesioned basolateral amygdalae (main effect of group: $F(1,9)=23.86$, $p=0.0009$; see Figure 3). The performance of the vehicle infused and the BLA lesioned animals differed both with respect to time (main effect of time block: $F(3,138)=9.57$, $p<0.0001$), and with respect to the type of choice learned (main effect of choice type: $F(5,138)=12.94$, $p<0.0001$). Once these group differences became apparent, the step down analyses allowed for the examination and comparison of each group, time block and choice type separately. With subsequent step down ANOVA's for each group (two way repeated measures ANOVA with block (4) and choice type (6) as within variables) it was found that the vehicle infused rats demonstrated significant improvement in choosing objects according to preference over the course of time (main effect of block: $F(3, 73)=12.2$; $p<0.0001$). On the other hand, rats with lesioned basolateral amygdala did not demonstrate learning over time (main effect of block: $F=1, 59$; $p=0.2618$).

One of the most important differences that can be seen between the performance of the vehicle infused and the BLA lesioned animals is the difference in learning distance relationships on a spectrum of affective values associated with objects over time. Specifically, the formation of preferences between a set of objects associated values that

are set closely on the valence continuum, such as the "Good vs. Bad" choice type, was compared with the preference formation between a set of objects associated values further spaced out, such as the "Best vs. Worst" choice type (two way repeated measures ANOVA with block (4) and proximity (2) as within variables for each group). There is a distinct dissociation between the performance of vehicle infused and BLA lesioned rats on these choice types (see Figure 3A and 3B). Rats with an intact BLA are able to learn to choose objects according to preference on both "Good vs. Bad" and the "Best vs. Worst" choices over time (vehicles, main effect of block: $F(3,361)=9.32$, $p<0.0001$; see Figure 3C). Rats with the lesioned BLA are not able to improve in their behavioral choices, "Good vs. Bad" and "Best vs. Worst" according to food preference over time (lesions, main effect of block: $F(3,295)=0.83$, $p=0.48$; see Figure 3D). However, while the lesioned rats show a marginal improvement in the "Best vs. Worst" choice type behavior (lesions, post hoc contrast comparison of the "adjacent" proximity variable by block: $F(1,295)=3.54$, $p=0.06$), they demonstrate no such improvement for the "Good vs. Bad" choice (lesions, post hoc contrast comparison of the "separate" proximity variable by block: $F(1,295)=0.023$, $p=0.88$; see Figure 3D).

This pattern of behavior is consistent with the different distances between the affective values on the valence continuum. While the "Good vs. Bad" choice type is not separated by any other food values, the "Best vs. Worst" choice type values are separated by the 25% sucrose pellet and the 0.002% Quinine HCl pellet values on the valence continuum.

In line with this method of choice data analysis, it was logical to compare the performance of both the experimental and the control groups of animals on all six

different types of choices according to the distance between associated values on the affect continuum (Adjacent vs. Separate value points, see Figure 4A and 4B). In support of this hypothesis, the vehicle infused rats performed significantly better on the choice types between objects associated with separate value points on the continuum ($F(1,32)=30.16$, $p<0.0001$). The BLA lesioned rats, on the other hand, did not differ in their choice performance according to the distance between the associated value of objects ($F(1, 63)=1.89$, $p=0.17$).

The six different choices between objects presented to rats in this experiment can also be grouped according to the appetitive or aversive qualities of the associated food pellets. In this case, the analysis is based on looking at choices between objects associated with opposite valence values (any positive vs. any negative food value), and the choices between objects associated with the similar valence values (positive vs. positive and negative vs. negative choice types). When the choices are grouped according to these categories (Within vs. Across valence), interesting similarities emerge between the experimental and the control groups of animals. The learning patterns for association of values with objects according to valence categories are different for both vehicle infused and the BLA lesioned rats (see Figure 4B and 4D). The vehicle infused rats are far better at learning the Across valence choice type responses than the Within valence choice types ($F(1,32)=50.08$, $p<0.0001$). However, the BLA lesioned rats are also better at learning the Across valence choices ($F(1,63)=6.58$, $p=0.01$).

The rats with intact BLA showed significant improvement over time in all choice types, with the possible exception of the “Best vs. Good” choice type, where the vehicle rats’ choice behavior was not significantly above chance over time. The rats’

performance shows that they were not able to learn to pick object 1 (100% sucrose pellet) over object 2 (25% sucrose pellet) by the end of training. The consumption tests (data omitted here) show that both lesioned and non-lesioned rats prefer whole sucrose pellets over 25% sucrose pellets every single time. However, the demands and conditions of the task may be such that it is exceedingly difficult for the rats to learn the distinction between the two positive objects. Alternatively, the 25% pellet may be "good enough" within the constraints of this task simply because it is not an aversive pellet. In an alternate, earlier version of this preference task, data were collected in the same manner as described above, with only distinction being that instead of the 25% sucrose pellet we used a 10% sucrose pellet. Also, the data was collected over 5 days of training for each object set instead of 6 days. It was evident from the data (omitted here) that rats improved performance on the choice between two "positive" objects. Furthermore, in an electrophysiological experiment based on this preference task, but executed with three objects instead of four, the rats were successfully able to learn the difference between the 25% sucrose and a 100% sucrose pellet in a similar choice format.

5. Discussion

When the behavior of both groups of rats, vehicle infused and lesioned, was examined carefully according to different parameters, several important points about the global role of the basolateral amygdala in affective associative learning could be discerned.

5.1 Are valence comparisons differentially disprupted according to separation on the valence continuum?

First, our findings in this task support the hypothesis that the BLA is undeniably important for the manifestation of learned affective goal directed choice behavior (Kesner et al., 1989; Holland et al., 2001, Balleine et al., 2003, Blundell et al., 2001, Schoenbaum et al., 1998, Chiba et al., 2002). This hypothesis also finds support in literature on human research that explores the role of the amygdala in affect, association and choice behavior using fMRI techniques (Arana et al., 2003; Coricelli et al., 2005).

Some of the other aspects of this task, such as the late emergence of instrumental learning, discussed in detail in chapter 2, did not depend so robustly on the BLA being intact, as does the behavior discussed here. Rats with an intact BLA demonstrated different rates and patterns of learning according to choice types, performing best on the choice types between objects that were both separate in distance on the value continuum as well as opposite in valence. These data support the idea that the dimension of valence is likely to be quite relevant to ongoing processing in amygdala circuitry.These results also support the theory of "pattern separation" for affective associations that was described and predicted by Kesner in relation to the specific role for the amygdala in learning and memory (Gilbert and Kesner, 2002). Several studies on magnitude of reinforcement in 8 arm maze place preference paradigms supported the role for the amygdala in being able to remember the location consistent with the size of reward (Kesner and Williams, 1995).

The results of the current experiment allow for the evolution of this hypothesis into one with a broader and better defined role for the BLA in affective associative

learning. The BLA participates in the linking of affective values with previously neutral visual objects, in the process integrating both the valence and the intensity of the associated affective values. There is a new study in human literature that supports this kind of integrative role for the amygdala (Winston et al., 2005). However, while the authors of the human study explored the interaction of various levels of intensity and gradations of affective valence of stimuli and their effects on the amygdalar activation during fMRI, the subjects in the study were never asked to choose between or associate previously neutral stimuli with these values (Winston et al., 2005).

The vehicle infused rats performed better on the choice types with more resolution (separated by other values on the valence continuum) than on the choice types with less resolution (see Figure 4A and 4B). Also, the vehicle infused rats were slower to improve and did not improve as much on the choice types with less resolution (see Figure 4A and 4B). These findings are fully consistent with the evidence presented above on the potential interaction between levels of intensity and valence, proposed by Winston et al, 2005.

Conversely, none of the BLA lesioned rats improved significantly on any of the choice types over time. However, the choice behavior of the BLA lesioned rats showed definite trends toward improvement that were evident by the last day of training, especially when compared to chance levels. The BLA lesioned rats showed the most improvement in choices between objects associated with opposite valence, positive versus negative, in a post-hoc analysis of choice types ($p=0.062$).

5.2 Does an absence of the putative "arousal stamp" provided by the BLA account for deficits in choice performance?

A number of studies from the McGaugh laboratory point to the basolateral amygdala as an important structure in the modulation of affective memory (Cahill and McGaugh, 1990; McGaugh et al., 1992; McGaugh et al., 1996). This modulation is demonstrated to occur through the additional arousal provided by the affective component of the stimulus. The integrity of the amygdala is proposed to be critical to the function of this arousal stamp, such that amygdala lesioned rats do not demonstrate the ordinary memory benefit provided by affectively laden material. The current study results are consistent with the BLA playing a role in modulating behavior based on affective associations. However, despite their overall poor performance, BLA lesioned rats eventually demonstrated some ability to appropriately choose between the two most arousing stimuli (positive vs. negative) presented in the experiment ($p=0.06$). In contrast, an experiment by Cahill, using a single positive and two gradations of negative reinforcements, found that the amygdala lesioned rats were not impaired in the memory of the positive and the lesser negative location (Cahill and McGaugh, 1990). (Here it is important to note that memory for our objects was not tested. In our study we did not test whether the rats remember the objects, but rather whether they can discern which of two objects leads to a better outcome.) Thus, this inconsistency with the present results could be due to a disctinction between memory for an item and the comparison of relevance between two items. Reliance of the present study on testing the ongoing formation of multiple associations provides a methodological difference that might also account for differences in interpretation. A majority of studies by McGaugh and colleagues assess the

involvement of the BLA in the consolidation of single events that are very high in intensity, which could potentially lead to the lack of effect when the same measure is used on events of lesser intensity.

5.3 Does some redundancy of function between BLA and OFC provide for some preserved learning, in the absence of BLA?

Electrophysiological studies from the Chiba laboratory showed that the single unit firing properties of the BLA cells assume selectivity very early in response to particular object – value pairings (Quinn et al., in preparation). The basolateral amygdala is bidirectionally interconnected with the orbitofrontal portion (OFC) of the prefrontal cortex, and there is evidence that the BLA is important for tuning the selectivity of neurons in the OFC (Schoenbaum et al., 2003). The OFC has been shown to be extremely important in overt emotional decision making and learning (Schoenbaum et al., 1999; Chiba et al., 2002; Bechara et al., 1999). It has also been shown that single cells in the BLA assume distinct firing patterns in response to stimuli of different affective values earlier than the cells in the OFC (Schoenbaum et al., 1999; Chiba et al., 2002).

For different choice types vehicle rats demonstrate proficiency at different rates across blocks of training. The choice type (best vs. worst) in which vehicle rats most rapidly demonstrate proficiency is the same choice type on which BLA lesioned rats demonstrate a trend toward learning, only at a later time point. Given an aspect of redundancy in the roles of amygdala and OFC in choice behavior (Bechara et al., 1999), it is possible that OFC can support this learning and simply does so more slowly without the input of the amygdala. The fact that the BLA lesioned rats in our experiment start

learning some of the more distinct choice types at a later time point is consistent with the OFC guiding this function without the input from the amygdala. Thus, it is entirely possible that learning of other choice types would emerge at even later time points, if BLA lesioned rats were trained extensively on a set of objects.

5.4 Is the amygdala specialized only for making associations between stimuli and negative outcomes?

In full support of the theories brought forth on the role of the basolateral amygdala in fear conditioning (Davis, 1992: LeDoux, 1992 ; Kim & Fanselow, 1992; Davis and Whalen, 2002), rats with BLA lesions were completely unable to improve their performance on the negative to negative (Bad vs. Worst) object choice type. That is, the BLA seems to bear crucial influence in learning to choose correctly the lesser of two evils, once those evils are associated with their evil values and are no longer neutral and innocent.

Why are the choice data presented in this chapter not merely a demonstration of rats learning something relative to how "bad" it is (Amaral et al., 1992; LeDoux et al., 1990)? Are the lesioned rats just unable to learn the significance and associability of "bad" objects? One of the answers lies in examining the rats' behavior while choosing between objects of relatively different acquired values. Rats with an intact BLA learn the difference between objects that are far away from each other on the "good to bad" spectrum, as well as the difference between objects that are relatively close to each other on this spectrum. While the rats with lesioned BLA demonstrate a trend toward learning the difference between objects that are far apart on the valence continuum, they are

absolutely not able to learn the difference between the objects that are close to each other on this continuum.

If the learning was occurring only on the level of "relative to bad", the rates and successes in learning these two different relationships should be comparable. Instead, what can be observed, is the effect of learning relative to any value on the continuum: not only to the negative, but to the relative degree, or gradation, of both positive and negative value(see Figure 3C, 4A, and 4B).

This result is closely aligned with the electrophysiological data from a similar experimental paradigm accomplished by Quinn et al., which shows that individual cells in the BLA acquire selective firing patterns in response to gradations of positive value associated objects, as well as with negative value associated objects (Quinn et al., in preparation).

Thus, the BLA seems to play a significant role in learning a spectrum of affective object – value associations, that are not linked with extreme levels of physiological arousal such as that elicited to cues in fear conditioning experiments (Iwata and LeDoux, 1988). Still, the data received from the delayed acute tone fear conditioning paradigm in the current study are consistent with the performance of BLA lesioned and intact rats on the preference formation task (see Figure 2).

5.5 Does the BLA support categorical learning (good vs. bad) or is there evidence that gradations of learning are differentiated?

Vehicle infused BLA rats, as opposed to the BLA lesioned rats learn the "Good v. Bad" choice over time, with a learning rate consistent with the other between valence

comparisons (see Figure 3C). Lesioned animals not only do not improve on this choice selection over time, there is a distinct learning rate difference in their performance of this particular choice from the other between valence choice types (see Figure 3D for a representative comparison with the "Best vs. Worst" choice type behavior). This serves as partial evidence of considerable disruption in the normal learning mechanisms that subserve affective associative learning. Furthermore, this is concrete evidence that the basolateral amygdala plays an important role in learning affective value relationships that include relationships between good and bad, as well as gradations of bad values, as opposed to learning distinct and independent value points (Kesner et al., 1989).

When the lesioned and the vehicle groups are examined separately, it becomes apparent that both groups are able to distinguish choice types based on the global valence difference of the value associations – either positive or negative. This is indicated in our analysis of the between and within valence object associations for each of the groups (see Figure 4B and 4D). Both the vehicle infused and the BLA lesioned rats demonstrate differences in how they learn the between and the within valence types of choices. This is irrespective of the fact that while vehicle infused rats improve over time, the lesioned rats do not.

However, when the lesioned and the vehicle groups are examined according to the choices they make between objects based on the associated value proximity on the valence continuum, a different picture emerges. While the vehicle infused rats are learning different types of choices at different rates – with separate and adjacent choice types clearly separating over time, the BLA lesioned rats demonstrate only partial separation of learning patterns for these disctinct types of choices.

These results indicate that the BLA influences the learning of relative values between objects disproportionately more so than the learning of global positive versus negative valence in the given experimental task. Even though the BLA lesioned rats are unable to improve their performance over time on the valence choices, their performance is still differentiated according to choice types in the between and within valence choice types. The BLA lesioned rats, thus, show covert learning for the difference between the positive and negative values of the objects, without the accompanying difference in overt behavior. This result is partially consistent with the role the amygdala exerts in choices between decks of different affective values in a gambling task in humans (Bechara et al., 1999). In this task, humans with amygdala damage were unable to choose advantageously based on the positive or aversive values associated with gambling cards. However, these humans also did not demonstrate any covert indication of knowing the difference between the cards, as measured by autonomic responses. Conversely, in our experiment the BLA lesioned animals show marginal improvement on more explicit choice types involving opposite valence comparisons.

The BLA lesioned rats seem unable to differentiate between the relative distances between objects associated with separate and adjacent values on the valence continuum. From this, it can be inferred that the BLA has a disproportionately larger role in learning relative values of neutral objects, versus acquiring the associations for negative or for positive values separately.

On the other hand, there are a number of neural structures discussed in the literature, other than the BLA, that are also important in appraising the negative or the positive qualities of stimuli. The central nucleus of the amygdala, as well as the BLA,

has been implicated in the learning of the aversive associations (Davis, 1992: LeDoux, 1992 ; Kim & Fanselow, 1992; Davis and Whalen, 2002). There are also a number of structures that are important through their interaction with the BLA, for learning the motivational and rewarding properties of stimuli, such as the ventral striatum and the striatopallidal circuitry (Everitt and Robbins, 1992, Alheid and Heimer, 1988, Parkinson et al., 2000; Everitt et al., 1989; Everitt et al., 1991). While the BLA is also important in these kinds of positive and negative appraisals, what ultimately separates it from the roles of these other structures is the importance that it assumes in the execution of goal directed choice behavior toward the objects.

The prefrontal cortex, which receives input from the BLA, has been shown to bear overt influence on choice behavior and decision making, evident in both animal and human literature (Schoenbaum et al., 1999; Chiba et al., 2002; Bechara et al., 1997; Bechara et al., 1999; Bechara et al., 2000; Parkinson et al., 2000; Rolls, 1999). However, as can be seen in the Bechara gambling task experiments with the amgydala and the ventromedial prefrontal cortex patients, the amygdala exerts powerful covert influence on the choice behavior exhibited on a task between stimuli with different values (Bechara et al., 1999). This is also consistent with the findings in the current studies, where the BLA lesioned animals demonstrated trends toward learning of across valence choice types. Putatively, if these animals were tested on the same object sets for a longer time, the prefrontal cortex influence on choice behavior would enable these rats to learn. This is also consistent with the electrophysiological single unit recording experiments in the Chiba laboratory, which emphasize the early emergent selectivity in the BLA cells to objects paired with distinct food values (Quinn et al., in preparation).

In conclusion, the basolateral amygdala plays a crucial role in learning both positive and aversive object – value associations. In this experiment we found that the integrity of the BLA was necessary for successful instrumental goal directed behavior to emerge with respect to the gradations of postitive as well as aversive values. The discussion of the other aspects of conditioning present in this task, with relation to the specific role of the BLA in instrumental learning, is continued in Chapter 2.

The BLA seems to play a more important role specifically in forming relative value associations, versus global good versus bad value associations. As there is some indication in the data that BLA rats begin to learn after extensive exposure to the same stimulus set, further research is needed to explicate precise nature of the BLA involvement in choice behavior over time and training.

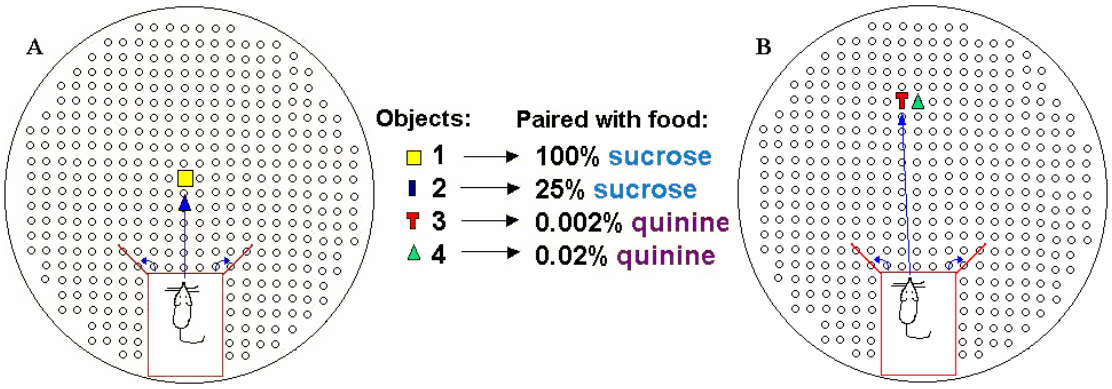


Figure 2.1 Open field behavioral apparatus Top view. Food stimuli are placed in the holes underneath objects. The board has netting underneath the holes where all types of food pellets were placed to avoid olfactory cueing. The object set-up shown reflects both a training trial (A) and a test trial (B) set up for the formation preference task.

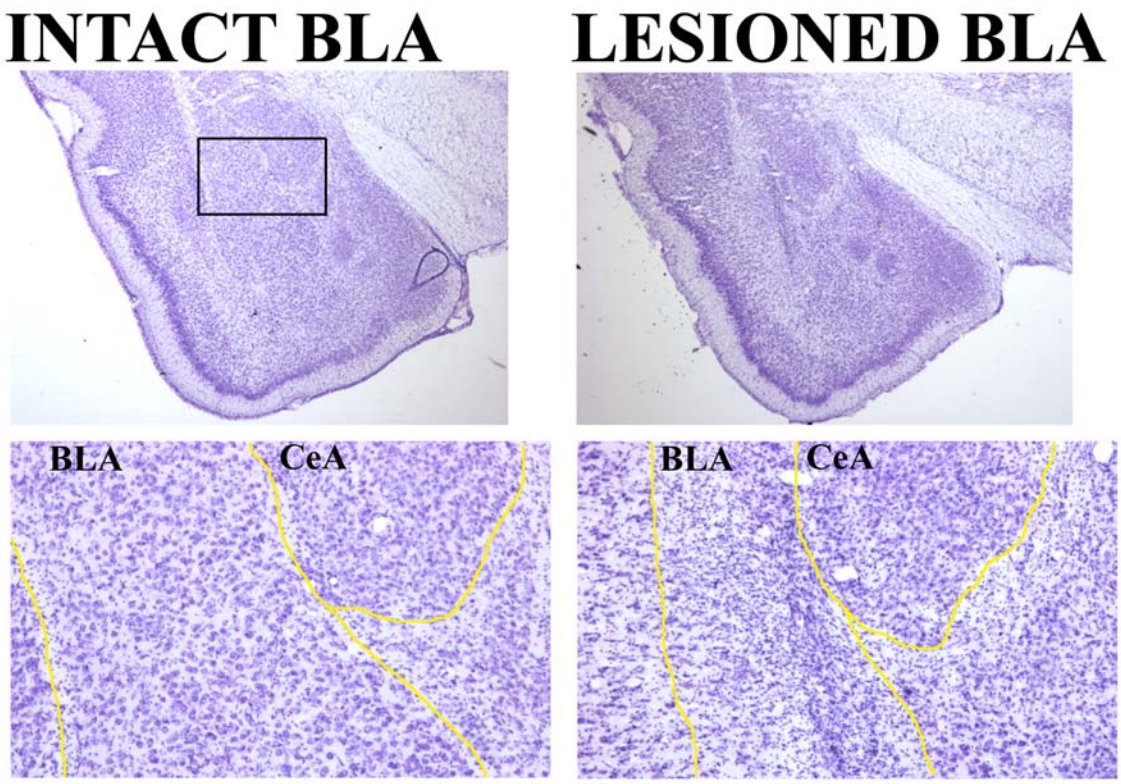


Figure 2.1.A Representative histology of the basolateral amygdala The sections on the left present a close up of the spared BLA pyramidal cells, representative for a vehicle infused animal. The sections on the right present a close up of post-excitatoxic gliosis, with sparing of some glia, but no pyramidal cells in a NMDA infused BLA, representative for a BLA lesioned animal.

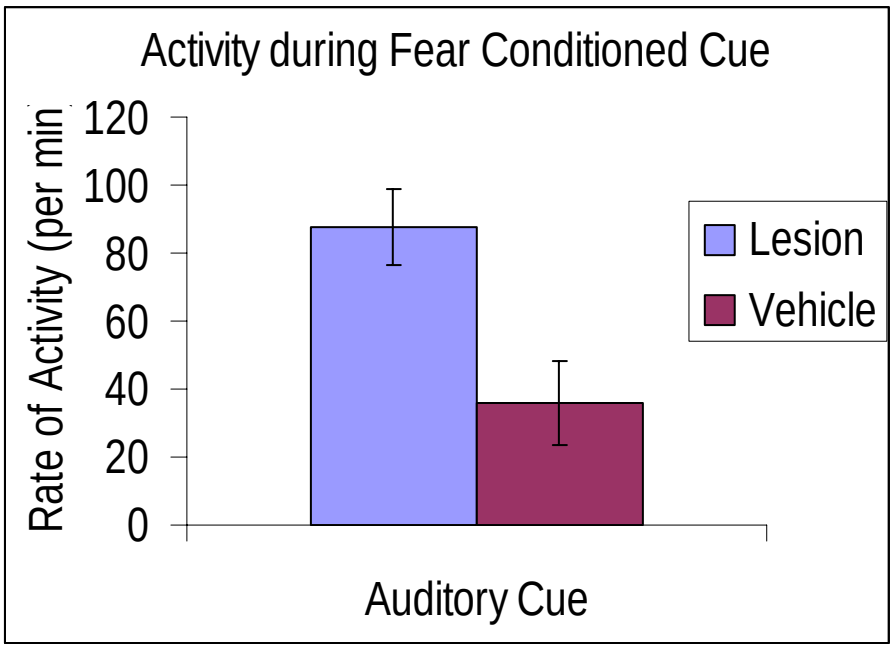


Figure 2.2 Fear conditioning delay-tone paradigm: the rate of activity of the BLA lesioned as compared to the vehicle infused rats during a fear conditioning test session. The rate of activity (Y-axis) is limited to the time the rats experienced a previously fear conditioned auditory cue (X-axis).

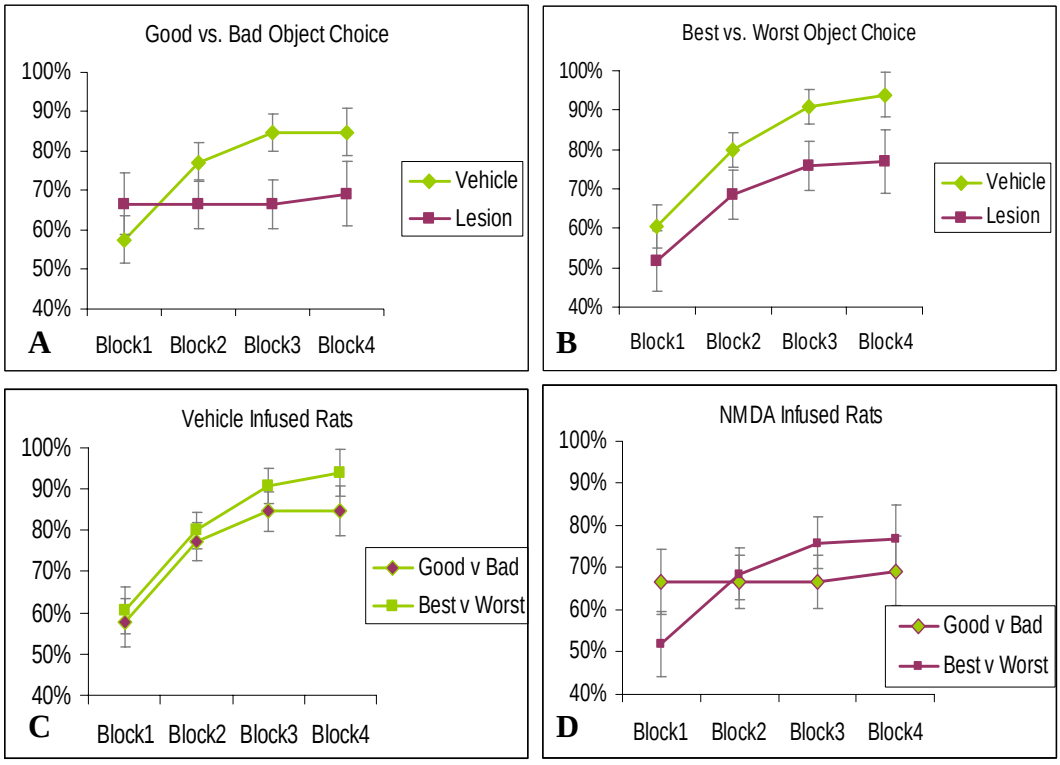


Figure 2.3 Averaged performance of the vehicle infused (n=6) and the BLA lesioned (n=5) rats on "choice" behavior over 4 time blocks of training (X-axis). Performance is based on food preference. The performance is shown as percent correct on a given "choice" or combination of choice types (Y-axis). This data has been averaged over 4 to 8 Lego object sets for each animal. Objects paired with food as follows: 1 (100% sucrose), 2 (10% sucrose), 3 (0.002% Quinine HCl), 4 (0.02% Quinine HCl). **(A)** The "Good v. Bad" choice type for the vehicle infused and the BLA lesioned rats, **(B)** The "Best v. Worst" choice type for the vehicle infused and the BLA lesioned rats, **(C)** The comparison between the "Good v. Bad" and the "Best v. Worst" choice type for the vehicle infused rats, **(D)** The comparison between the "Good v. Bad" and the "Best v. Worst" choice type for the BLA lesioned rats.

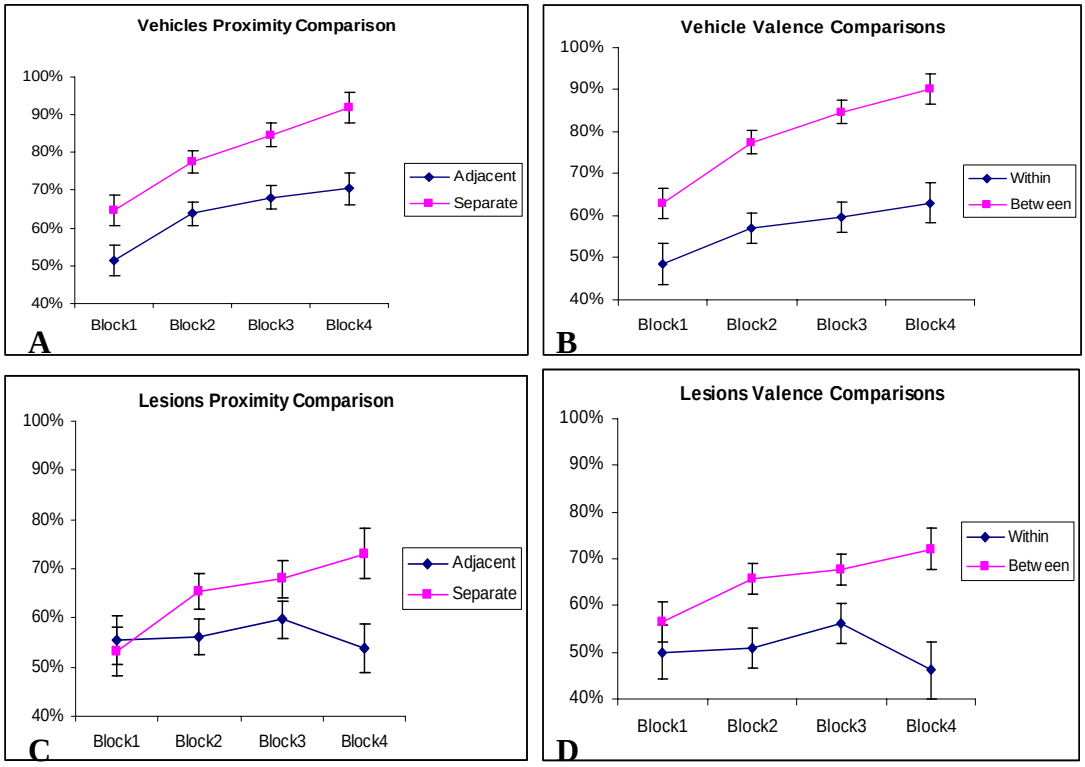


Figure 2.4 Averaged performance of the vehicle infused (n=6) and the BLA lesioned (n=5) rats on categorically grouped choice-types behavior over 4 time blocks of training (X-axis). Performance is based on food preference. The performance is shown as percent correct on a given "choice" or combination of choice types (Y-axis). This data has been averaged over 4 to 8 Lego object sets for each animal. Objects paired with food as follows: 1 (100% sucrose), 2 (10% sucrose), 3 (0.002% Quinine HCl), 4 (0.02% Quinine HCl). **(A)** Performance of the vehicle infused rats on "Adjacent" choice types (including "Best v. Good", "Good v. Bad", and "Bad v. Worst" choices) compared with "Separate" choice types (including "Best v. Bad", "Good v. Worst", and "Best v. Worst" choices), **(B)** Performance of the vehicle infused rats on "Within" choice types (including "Best v. Good" and "Bad v. Worst" choices) compared with "Between" choice types (the other four object combinations), **(C)** and **(D)** are the same comparisons for the BLA lesioned rats.

6. References

Alheid, G. F., & Heimer, L. (1988). New perspectives in basal forebrain organization of special relevance for neuropsychiatric disorders: the striatopallidal, amygdaloid, and corticopetal components of substantia innominata. Neuroscience, 27(1), 1-39.

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.

Amaral DG, Behniea H, Kelly JL., Topographic organization of projections from the amygdala to the visual cortex in the macaque monkey, Neuroscience. 2003;118(4):1099-120.

Arana FS, Parkinson JA, Hinton E, Holland AJ, Owen AM, Roberts AC. Dissociable contributions of the human amygdala and orbitofrontal cortex to incentive motivation and goal selection. J Neurosci. 2003 Oct 22;23(29):9632-8.

Armony, J. L., Servan-Schreiber, D., Cohen, J. D., & LeDoux, J. E. (1995). An anatomically constrained neural network model of fear conditioning. Behav Neurosci, 109(2), 246-257.

Arnold, M. B. (1969). Human emotion and action. In T. Mischel (Ed.), Human action:Conceptual and empirical issues (pp. 167-197). New York: Academic Press.

Balleine BW. Neural bases of food-seeking: Affect, arousal and reward in corticostriatolimbic circuits. Physiol Behav. 2005 Dec 15;86(5):717-30. Epub 2005 Oct 27

Balleine, B.W., Leibeskind, J.C. and Dickinson, A.: Effects of cell body lesions of the Basolateral Amygdala on instrumental conditioning. Soc. Neuroscience Abstract 1997, 23, 786.

Balleine, B. W., Killcross, A. S., & Dickinson, A. (2003). The effect of lesions of the basolateral amygdala on instrumental conditioning. J Neurosci, 23(2), 666-675.

Baxter, M. G., & Murray, E. A. (2002). The amygdala and reward. Nat Rev Neurosci, 3(7), 563-573.

Baxter, M. G., Parker, A., Lindner, C. C., Izquierdo, A. D., & Murray, E. A. (2000). Control of response selection by reinforcer value requires interaction of amygdala and orbital prefrontal cortex. J Neurosci, 20(11), 4311-4319.

Bechara A, Tranel D, Damasio H. Characterization of the decision-making deficit of patients with ventromedial prefrontal cortex lesions. Brain. 2000 Nov;123 (Pt 11):2189-202.

Bechara A, Damasio H, Damasio AR, Lee GP. Different contributions of the human amygdala and ventromedial prefrontal cortex to decision-making. J Neurosci. 1999 Jul 1;19(13):5473-81.

Bechara A, Damasio H, Tranel D, Damasio AR. advantageously before knowing the advantageous strategy. Science. 1997 Feb 28;275(5304):1293-5.

Berdel, B., Morys, J., & Maciejewska, B. (1997). Neuronal changes in the basolateral complex during development of the amygdala of the rat. Int J Dev Neurosci, 15(6), 755-765.

Berridge, K. C. (2000). Measuring hedonic impact in animals and infants: microstructure of affective taste reactivity patterns. Neurosci Biobehav Rev, 24(2), 173-198.

Blundell, P., Hall, G., & Killcross, S. (2001). Lesions of the basolateral amygdala disrupt selective aspects of reinforcer representation in rats. J Neurosci, 21(22), 9018-9026.

Brown, V. J., Latimer, M. P., & Winn, P. (1996). Memory for the changing cost of a reward is mediated by the sublenticular extended amygdala. Brain Res Bull, 39(3), 163-170.

Cador, M., Robbins, T. W., & Everitt, B. J. (1989). Involvement of the amygdala in stimulus-reward associations: interaction with the ventral striatum. Neuroscience, 30(1), 77-86.

Cahill, L., Babinsky, R., Markowitsch, H. J., & McGaugh, J. L. (1995). The amygdala and emotional memory. Nature, 377(6547), 295-296.

Cahill, L., Haier, R. J., Fallon, J., Alkire, M. T., Tang, C., Keator, D., Wu, J., & McGaugh, J. L. (1996). Amygdala activity at encoding correlated with long-term, free recall of emotional information. Proc Natl Acad Sci U S A, 93(15), 8016-8021.

Cahill L, McGaugh JL. Amygdaloid complex lesions differentially affect retention of tasks using appetitive and aversive reinforcement. Behav Neurosci. 1990 Aug;104(4):532-43.

Caldji, C., Tannenbaum, B., Sharma, S., Francis, D., Plotsky, P. M., & Meaney, M. J. (1998). Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. Proc Natl Acad Sci U S A,

95(9), 5335-5340.

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 conditioned stimuli. J Neurosci, 15(3 Pt 2), 2301-2311.

Charney, Dennis S.; Bremner, J. Douglas; Redmond, D. Eugene,. (1995). Noradrenergic neural substrates for anxiety and fear: Clinical associations based on preclinical research. Bloom, F. E. Kupfer, D. J., In: Psychopharmacology: The fourth generation of progress. Raven Press ; 1185 Avenue of the Americas, New York, New York 10036-2806, USA, 1995. 387-395.

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. J Neuropsychiatry Clin Neurosci, 9(3), 382-402.

Davis, M. (1998). Are different parts of the extended amygdala involved in fear versus anxiety? Biol Psychiatry, 44(12), 1239-1247.

Davis, M., & Whalen, P. J. (2001). The amygdala: vigilance and emotion. Mol Psychiatry, 6(1), 13-34.

Derryberry, Douglas & Tucker, Don M. (1994). Motivating the focus of attention. In: Paula M. Niedenthal, Ed; Shinobu Kitayama, Ed; et al. The heart's eye: Emotional influences in perception and attention.. Academic Press, Inc: San Diego, CA, USA, p. 167-196 of xiv, 289pp.

Everitt, B. J., Cador, M., & Robbins, T. W. (1989). Interactions between the amygdala and ventral striatum in stimulus-reward associations: studies using a second-order schedule of sexual reinforcement. Neuroscience, 30(1), 63-75.

Everitt, B. J., Dickinson, A., & Robbins, T. W. (2001). The neuropsychological basis of addictive behaviour. Brain Res Brain Res Rev, 36(2-3), 129-138.

Everitt, B. J., Morris, K. A., O'Brien, A., & Robbins, T. W. (1991). The basolateral amygdala-ventral striatal system and conditioned place preference: further evidence of limbic-striatal interactions underlying reward-related processes. Neuroscience, 42(1), 1-18.

Everitt, B.J., Robbins, T,W. (1992). Amygdala-ventral striatal interactions and reward-related processes. In The Amygdala. Edited by JP Aggleton. New York City: Wiley-Liss; 401-430.

Everitt BJ, Cardinal RN, Hall J, Parkinson J, Robbins T. (2000). Differential involvement of amygdala subsystems in appetitve conditioning and drug addiction. In The Amygdala. Edited by JP Aggleton. New York City: Oxford University Press; 353-381.

Fallon, J.H. & Ciofi, P. 1992 Distribution of monoamines within the amygdala. In: Aggleton, J. P, ed. The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction. Wiley-Liss, New York, pp. 97-114.

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. Behav Neurosci, 108(1), 210-212.

Ferry, B., Roozendaal, B., & McGaugh, J. L. (1999a). Involvement of alpha1-adrenoceptors in the basolateral amygdala in modulation of memory storage. Eur J Pharmacol, 372(1), 9-16.

Ferry, B., Roozendaal, B., & McGaugh, J. L. (1999b). Basolateral amygdala noradrenergic influences on memory storage are mediated by an interaction between beta- and alpha1-adrenoceptors. J Neurosci, 19(12), 5119-5123.

Ferry, B., Roozendaal, B., & McGaugh, J. L. (1999c). Role of norepinephrine in mediating stress hormone regulation of long-term memory storage: a critical involvement of the amygdala. Biol Psychiatry, 46(9), 1140-1152.

Freese JL, Amaral DG., The organization of projections from the amygdala to visual cortical areas TE and V1 in the macaque monkey, J Comp Neurol. 2005 Jun 13;486(4):295-317.

Fuchs, R. A., Weber, S. M., Rice, H. J., & Neisewander, J. L. (2002). Effects of excitotoxic lesions of the basolateral amygdala on cocaine-seeking behavior and cocaine conditioned place preference in rats. Brain Res, 929(1), 15-25.

Furedy, J. J. (1992). Reflections on human Pavlovian decelerative heart-rate conditioning with negative tilt as US: alternative approaches. Integr Physiol Behav Sci, 27(4), 347-355.

Gallagher, M., & Chiba, A. A. (1996). The amygdala and emotion. Curr Opin Neurobiol, 6(2), 221-227.

Gallagher, M., Kapp, B. S., & Pascoe, J. P. (1982). Enkephalin analogue effects in the amygdala central nucleus on conditioned heart rate. Pharmacol Biochem Behav, 17(2), 217-222.

Gilbert PE, Kesner RP. 2002. The amygdala but not the hippocampus is involved in pattern separation based on reward value. Neurobiol Learn Mem May;77(3):338-53)

Gottfried JA, O'Doherty J, Dolan RJ. Encoding predictive reward value in human amygdala and orbitofrontal cortex. Science. 2003 Aug 22;301(5636):1104-7.

Hamann, S. B., Ely, T. D., Grafton, S. T., & Kilts, C. D. (1999). Amygdala activity related to enhanced memory for pleasant and aversive stimuli. Nat Neurosci, 2(3), 289-293.

Hatfield, T., Han, J. S., Conley, M., Gallagher, M., & Holland, P. (1996). Neurotoxic lesions of basolateral, but not central, amygdala interfere with Pavlovian second-order conditioning and reinforcer devaluation effects. J Neurosci, 16(16), 5256-5265.

Heimer, L., Alheid, G. F., de Olmos, J. S., Groenewegen, H. J., Haber, S. N., Harlan, R. E., & Zahm, D. S. (1997). The accumbens: beyond the core-shell dichotomy. J Neuropsychiatry Clin Neurosci, 9(3), 354-381.

Hiroi, N., & White, N. M. (1991). The lateral nucleus of the amygdala mediates expression of the amphetamine-produced conditioned place preference. J Neurosci, 11(7), 2107-2116.

Holland, P. C., Chik, Y., & Zhang, Q. (2001). Inhibitory learning tests of conditioned stimulus associability in rats with lesions of the amygdala central nucleus. Behav Neurosci, 115(5), 1154-1158.

Iwata J, LeDoux JE. Dissociation of associative and nonassociative concomitants of classical fear conditioning in the freely behaving rat. Behav Neurosci. 1988 Feb;102(1):66-76

Izquierdo A, Murray EA. Combined unilateral lesions of the amygdala and orbital prefrontal cortex impair affective processing in rhesus monkeys. J Neurophysiol. 2004 May;91(5):2023-39. Epub 2004 Jan 7

Kesner, R. P., Berman, R. F., & Tardif, R. (1992). Place and taste aversion learning: role of basal forebrain, parietal cortex, and amygdala. Brain Res Bull, 29(3-4), 345-353.

Kesner, R. P., Walser, R. D., & Winzenried, G. (1989). Central but not basolateral

amygdala mediates memory for positive affective experiences. Behav Brain Res, 33(2), 189-195.

Kesner, R. P., & Williams, J. M. (1995). Memory for magnitude of reinforcement: dissociation between the amygdala and hippocampus. Neurobiol Learn Mem, 64(3), 237-244.

Killcross, S., Robbins, T. W., & Everitt, B. J. (1997). Different types of fear-conditioned behaviour mediated by separate nuclei within amygdala. Nature, 388(6640), 377-380.

Kluver, H., & Bucy, P. C. (1997). Preliminary analysis of functions of the temporal lobes in monkeys. 1939. J Neuropsychiatry Clin Neurosci, 9(4), 606-620.

Koob, G. F. (1999). Corticotropin-releasing factor, norepinephrine, and stress. Biol Psychiatry, 46(9), 1167-1180.

Koob, G. F., & Le Moal, M. (2001). Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology, 24(2), 97-129.

Lang, P. J. (1995). The emotion probe. Studies of motivation and attention. Am Psychol, 50(5), 372-385.

LeDoux, J. E. (1993). Emotional memory systems in the brain. Behav Brain Res, 58(1-2), 69-79.

LeDoux, J. E., Cicchetti, P., Xagoraris, A., & Romanski, L. M. (1990). The lateral amygdaloid nucleus: sensory interface of the amygdala in fear conditioning. J Neurosci, 10(4), 1062-1069.

LeDoux, J. E., Iwata, J., Pearl, D., & Reis, D. J. (1986). Disruption of auditory but not visual learning by destruction of intrinsic neurons in the rat medial geniculate body. Brain Res, 371(2), 395-399.

Liang, KC. And Chiang, TC. (1994) Locus coeruleus infusion of clonidine impaired retention and attenuated memory enhancing effects of epinephrine. Society for Neuroscience Abstracts, 20, 153.

Malkova, L., Gaffan, D., & Murray, E. A. (1997). Excitotoxic lesions of the amygdala fail to produce impairment in visual learning for auditory secondary reinforcement but interfere with reinforcer devaluation effects in rhesus monkeys. J Neurosci, 17(15), 6011-6020.

McDonald, A. J. (1998). Cortical pathways to the mammalian amygdala. Prog Neurobiol, 55(3), 257-332.

McDonald, A. J., & Mascagni, F. (1996). Cortico-cortical and cortico-amygdaloid projections of the rat occipital cortex: a Phaseolus vulgaris leucoagglutinin study. Neuroscience, 71(1), 37-54.

McDonald, R. J., & White, N. M. (1995). Information acquired by the hippocampus interferes with acquisition of the amygdala-based conditioned-cue preference in the rat. Hippocampus, 5(3), 189-197.

McEwen, B. S. (2000). Allostasis and allostatic load: implications for neuropsychopharmacology. Neuropsychopharmacology, 22(2), 108-124.

McGaugh, J. L., Cahill, L., & Roozendaal, B. (1996). Involvement of the amygdala in memory storage: interaction with other brain systems. Proc Natl Acad Sci U S A, 93(24), 13508-13514.

McGaugh, J.L., Introini-Collison ,I.B., Cahill, L., Kim, M., Liang, K.C.(1992). Involvement of the amygdala in neuromodulatory influences on memory storage. In The Amygdala. Edited by JP Aggleton. New York City: Wiley-Liss; (1992):431-452.

McGaugh, J.L., Ferry B, Vazdarjanova, A, Roozendaal, B. (2000). Amygdala: role in modulation of memory storage. In The Amygdala. Edited by JP Aggleton. New York City: Oxford University Press, 391-412.

Miyashita, T., & Williams, C. L. (2002). Glutamatergic transmission in the nucleus of the solitary tract modulates memory through influences on amygdala noradrenergic systems. Behav Neurosci, 116(1), 13-21.

Ono, T., Nishijo, H., & Uwano, T. (1995). Amygdala role in conditioned associative learning. Prog Neurobiol, 46(4), 401-422.

Parkinson, J. A., Cardinal, R. N., & Everitt, B. J. (2000). Limbic cortical-ventral striatal systems underlying appetitive conditioning. Prog Brain Res, 126, 263-285.

Parkinson, J. A., Crofts, H. S., McGuigan, M., Tomic, D. L., Everitt, B. J., & Roberts, A. C. (2001). The role of the primate amygdala in conditioned reinforcement. J Neurosci, 21(19), 7770-7780.

Pitkanen, A., Savander, V., & LeDoux, J. E. (1997). Organization of intra-amygdaloid circuitries in the rat: an emerging framework for understanding functions of the amygdala. Trends Neurosci, 20(11), 517-523.

Pitkanen, A., Stefanacci, L., Farb, C. R., Go, G. G., LeDoux, J. E., & Amaral, D. G. (1995). Intrinsic connections of the rat amygdaloid complex: projections

originating in the lateral nucleus. J Comp Neurol, 356(2), 288-310.

Price JL, Fokje TR, Amaral, DG. (1987) The limbic region. II: The amygdaloid complex. In Handbook of Chemical Neuroanatomy, edited by Bjorklund, Hokfelt, Swanson. Elsevier Science Publishers. 279-388.

Quirarte, G. L., Roozendaal, B., & McGaugh, J. L. (1997). Glucocorticoid enhancement of memory storage involves noradrenergic activation in the basolateral amygdala. Proc Natl Acad Sci U S A, 94(25), 14048-14053.

Ragozzino, M. E., & Kesner, R. P. (1999). The role of the agranular insular cortex in working memory for food reward value and allocentric space in rats. Behav Brain Res, 98(1), 103-112.

Romanski, L. M., & LeDoux, J. E. (1992). Equipotentiality of thalamo-amygdala and thalamo-cortico-amygdala circuits in auditory fear conditioning. J Neurosci, 12(11), 4501-4509.

Roozendaal, B. (2000). 1999 Curt P. Richter award. Glucocorticoids and the regulation of memory consolidation. Psychoneuroendocrinology, 25(3), 213-238.

Roozendaal, B., Williams, C. L., & McGaugh, J. L. (1999). Glucocorticoid receptor activation in the rat nucleus of the solitary tract facilitates memory consolidation: involvement of the basolateral amygdala. Eur J Neurosci, 11(4), 1317-1323.

Rosen, J. B., Hitchcock, J. M., Miserendino, M. J., Falls, W. A., Campeau, S., & Davis, M. (1992). Lesions of the perirhinal cortex but not of the frontal, medial prefrontal, visual, or insular cortex block fear-potentiated startle using a visual conditioned stimulus. J Neurosci, 12(12), 4624-4633.

Rolls, E.T., (1999). The Brain and Emotion. Oxford University Press, Oxford, England.

Salinas, J. A., Dickinson-Anson, H., & McGaugh, J. L. (1994). Midazolam administered to rats induces anterograde amnesia for changes in reward magnitude. Behav Neurosci, 108(6), 1059-1064.

Salinas, J. A., & McGaugh, J. L. (1995). Muscimol induces retrograde amnesia for changes in reward magnitude. Neurobiol Learn Mem, 63(3), 277-285.

Salinas, J. A., Parent, M. B., & McGaugh, J. L. (1996). Ibotenic acid lesions of the amygdala basolateral complex or central nucleus differentially effect the response to reductions in reward. Brain Res, 742(1-2), 283-293.

Savander, V., LeDoux, J. E., & Pitkanen, A. (1996). Topographic projections from the periamygdaloid cortex to select subregions of the lateral nucleus of the amygdala

in the rat. Neurosci Lett, 211(3), 167-170.

Schoenbaum, G., Chiba, A. A., & Gallagher, M. (1999). Neural encoding in orbitofrontal cortex and basolateral amygdala during olfactory discrimination learning. J Neurosci, 19(5), 1876-1884.

Schoenbaum G, Setlow B, Saddoris MP, Gallagher M. Encoding predicted outcome and acquired value in orbitofrontal cortex during cue sampling depends upon input from basolateral amygdala. Neuron. 2003 Aug 28;39(5):855-67.

Schultz, W., Tremblay, L., & Hollerman, J. R. (2000). Reward processing in primate orbitofrontal cortex and basal ganglia. Cereb Cortex, 10(3), 272-284.

Setlow, B., Holland, P. C., & Gallagher, M. (2002). Disconnection of the basolateral amygdala complex and nucleus accumbens impairs appetitive pavlovian second-order conditioned responses. Behav Neurosci, 116(2), 267-275.

Stefanacci L, Farb CR, Pitkanen A, Go G, LeDoux JE, Amaral DG. Projections from the lateral nucleus to the basal nucleus of the amygdala: a light and electron microscopic PHA-L study in the rat. J Comp Neurol. 1992 Sep 22;323(4):586-601.

Swanson, L. W., & Petrovich, G. D. (1998). What is the amygdala? Trends Neurosci, 21(8), 323-331.

Weiskrantz, L. (1956). Behavioral changes associated with ablation of amygdaloid complex in monkeys. Journal of Comparative and Physiological Psychology 49, 381-391.

Whalen, P. J., Rauch, S. L., Etcoff, N. L., McInerney, S. C., Lee, M. B., & Jenike, M. A. (1998). Masked presentations of emotional facial expressions modulate amygdala activity without explicit knowledge. J Neurosci, 18(1), 411-418.

White, N. M. (1989). Reward or reinforcement: what's the difference? Neurosci Biobehav Rev, 13(2-3), 181-186.

White NM, McDonald RJ. Acquisition of a spatial conditioned place preference is impaired by amygdala lesions and improved by fornix lesions. Behav Brain Res. 1993 Jun 30;55(2):269-81

Winston JS, Gottfried JA, Kilner JM, Dolan RJ. Integrated neural representations of odor intensity and affective valence in human amygdala. J Neurosci. 2005 Sep 28;25(39):8903-7.

III

Basolateral Amygdala in instrumental learning

1. Abstract

The role of the basolateral amygdala in instrumental learning is multidimensional and complex, as can be inferred from the large and diverse body of literature on the subject. The last chapter explored how the BLA contributes to the elicitation of overt choice behavior when picking between pairs of affectively significant visual objects. This chapter focuses on the role of the BLA in the elicitation of specific types of instrumental responses to individual visual stimuli. These responses, both covert and overt in nature, emerge over the course of learning the affective values associated with the stimuli. The basolateral amygdala significantly contributes to the rats behavior on approach/avoid, or "microchoice", responses to objects. The BLA also contributes to systematic differences in approach latencies to reach the objects, based on gradations in positive and negative associated values. The results demonstrate that the basolateral amygdala has a distinct role specifically in the early learning of these responses. The implications of these results for the incentive learning process are discussed here with respect to motivated motor action planning and execution, to the preferential visual processing of salient stimuli in the environment, and to the neural structures and circuitries likely to subserve early as opposed to later stages of instrumental learning.

2. Introduction

It is evolutionarily advantageous to devote more neural resources to processing emotionally salient stimuli in the environment. For example, the visual symbol of the golden arches is no longer neutral for nearly anyone in the industrialized society. Depending on the affective state associated with McDonalds, this relevant stimulus can serve to stimulate approach or avoidance behavior. The specificity of approach or avoidance behavior toward stimuli in the environment underlies most of the instrumental learning processing that allows organisms to exert a degree of control over their environments.

An established line of research using both animal and human models has demonstrated that the basolateral complex of the amygdala (BLA), lies at the heart of the neural circuitry important for the regulation of these types of instrumental responses to salient stimuli (Killcross et al., 1998; McGaugh et al., 1992; Balleine et al., 1997; Winston et al., 2005; Balleine, 2005; Everitt et al., 1989; Wang et al., 2005).

The amygdala is also necessary for emotional face processing and is required for top-down modulation of behaviorally relevant and irrelevant stimuli (Ishai et al., 2004a; Ishai et al., 2004b; Vuilleumier et al., 2004). Basal and lateral parts of the amygdala receive input from many sensory cortical areas, including visual and gustatory, making it an ideal location for the association of food stimuli with visual stimuli (Swanson and Petrovich, 1998, Heimer et al., 1997, Pitkanen et al., 1995; McDonald, 1998; McDonald and Mascagni, 1996; see Chapter 1 for review).

The aspects of the experiment discussed herein focus on the evaluation of instrumental response formation in rats to novel visual stimuli that are repeatedly paired with differential food reinforcements. The use of value gradations of the food pellets

spanning the valence continuum from positive to negative, provide a range of both sensory and motivational (intensity) measures of stimuli that may help clarify the role of the BLA in incentive learning.

An appraisal of a visual object as either rewarding or aversive confers affective significance to this object, thus increasing its visual salience in the environment. This evaluation enables further cognitive processing, such as action planning and execution. The goal of this experiment was to elucidate how processing in the basolateral amygdala contributes to the interaction of positive and aversive affective states and visual associative learning to produce appropriate instrumental behavioral output.

There has been a lot of controversy in the literature regarding the nature of the specific contribution of the BLA to instrumental learning. There is support for the hypothesis that the BLA helps to encode values with respect to the sensory properties of the reinforcements during instrumental learning (Balleine et al., 1997; Corbit and Balleine, 2005). There is also extensive support for the role of the BLA in early memory modulation for sensory events linked with a high degree of physiological arousal, or intensity (McGaugh et al., 1992; Williams and McGaugh, 1993; Cahill at al., 1995). Additionally, both animal and human studies have provided support for the theory that the BLA is only coding stimuli with respect to the negative affective values (Davis, 1992: LeDoux, 1992 ; Fanselow and Kim, 1992; Davis and Whalen, 2002).

Conversely, there are also a number of studies that explore the BLA's involvement in modulating instrumental responses in appetitively motivated paradigms (Everitt et al., 1989; Everitt and Robbins, 1992; Everitt et al., 2000; Everitt et al., 2001; Balleine, 2005). Most of the appetitive instrumental conditioning paradigms that

illuminate the role of the BLA also implicate the interaction of the BLA with the ventral striatal and the striatopallidal systems involved in incentive learning (Everitt et al., 1989; Everitt and Robbins, 1992; Everitt et al., 2000; Everitt et al., 2001).

In addition, there are a number of research studies demonstrating that the amygdala plays a significant role in the top-down modulation of visual sensory systems during learning (Ishai et al., 2004a; Ishai et al., 2004b; Vuilleumier et al., 2004; Tabbert et al., 2005).

The involvement of the visual system in stimulus detection is modulated by many different cognitive processes, including attention and emotion. There is neuroanatomical evidence for subcortical modulation of processing in the visual cortex of rats, as well as primates and humans (Pegna et al., 2005; Dringenberg et al., 2004). Extensive neural projections from the basal nucleus of the amygdala to V1 and V2 in monkeys and to occipital area 1 in rats show that emotion and motivation may play a significant role in modulating perception at the earliest levels (Amaral et al., 2003; Freese and Amaral, 2005; Price et al., 1987). It is very likely that learning about the relevance of a visual stimulus leads to increased processing of this stimulus at some of the earliest stages in the visual system, such as V1 and V2. In studies with humans, increased emotion-related activation of the extended visual cortex was not only observed during actual emotional stimulation, but also during mere anticipation of an unpleasant visual stimulus (Ueda et al., 2003).

A novel behavioral task, discussed in detail in Chapter 1, was used here to evaluate the role of the BLA in formation of approach and avoid behaviors to previously neutral visual objects paired with different food values over time. Learning the

association between these stimuli results in the formation of an affective preference scale for the objects that can be measured by analyzing the behavior of the rats. We effectively demonstrated that the BLA is important in early instrumental learning of affective associations, and that processing in the BLA also affects both early and late aspects of instrumental learning, as shown in latencies to approach both preferred and non-preferred objects.

In light of the results from this experiment, the BLA plays multiple roles, coding many different aspects of instrumental learning. Overall, the findings from the current study support the hypothesis that the BLA is important for the early instrumental learning and appropriate behavioral response to affectively significant stimuli (Everitt and Robbins, 1992; Parkinson et al., 2000; Corbit and Balleine, 2005; Balleine, 2005).

3. Materials and methods

The "Methods and Materials" section of Chapter 1 discusses in detail the experimental set up, the task, the subjects, the neural manipulations of the BLA, and the behavioral training involved. Part of the behavioral training, relevant for the discussion in this chapter, involved extensive training on several sets of objects that entailed approaching each object individually and pushing it over in order to obtain the food pellet underneath. These behaviors were digitally recorded and analyzed separately for the approach ("Go") and avoid ("No Go") responses, as well as for the latency of approach in case of a "Go" response.

3.1 "Microchoice" scoring

All of the rats' performance in this experiment was recorded with a stationary digital camera set up above the platform. The videos were analyzed for approach/avoid or "go" – "no-go" behavior of the rats toward Lego objects during training trials. If the rat pushed or nudged the object after approaching it, this was scored as a "go". Alternatively, if the object remained unmoved, this was scored as a "no-go". The "microchoice" analysis also involved scoring of "go" – "no-go" behavior toward the pellets under the objects. If a rat made a "go" toward the object, it would be further determined if the rat stuck its nose into the hole, which was scored as a "go" to the pellet, or if the rat kept its head level with the object, which was scored as a "no-go" to the pellet.

3.2 Latency Scoring

The videos were separately analyzed to determine the time it took for the rats to get from the start box to the objects during training trials. These latencies were determined by advancing the videos frame by frame until the rat reached the first row of holes from the edge of the box. This was then recorded as the start time. The time point when the rat pushed or nudged the object from its starting position was also determined by advancing the video frame by frame. This was then recorded as the end point time. The difference between these time points was calculated as the latency. The latencies were scored for each training trial and averaged for each of the four objects for a given day of training. Because of the early data showing very early effects of learning, the latencies from the first day of a given object set were split into two blocks: the first block of latencies was taken from the training trials before the first test trial, and the second

block of latencies was taken from the training trials after the first test trial. To look at the BLA effects on late instrumental learning, for the final day of training of a given object set, the latencies were averaged over the entire day of training.

3.3 Statistical Analysis

All of the animals included in the data analyses for the BLA lesion group and for the vehicle infused group are the same animals included in these group analyses in Chapter 1. Microchoices and latencies were averaged by object for a given block of data and were subsequently analyzed using a three way repeated measures ANOVA – two within variables (time blocks of training (3), and the objects (4)), one between variable (lesion/vehicle group). Subsequent step down ANOVAs and post-hoc comparisons were performed on variables with significant main effects. The latency data was analyzed separately for each time block of learning, with step-down analyses for the control group and experimental group performed separately. The microchoice data was first analyzed as outlined above, and then separate analyses were performed on correct responses for the negative objects (the "bad" and the "worst" reinforcement paired objects). The correct "go"/"no-go" responses to the objects were analyzed using a three way repeated measures ANOVA with group as between variable (2), and blocks of training (3), and the objects (2) as within variables. Subsequent step down analyses and were used to analyze the performance of the groups on microchoices and latencies separately.

4. Results

4.1 Microchoice Data

In the analysis of approach/avoid microchoice data in this study, it was found that the rats with intact basolateral amygdalae demonstrated a significantly different distribution of responses toward the objects when compared with the BLA lesioned animals ($F(1,9)=55.64$, $p<0.0001$; see Figure 1 and Figure 2). The distributions of the microchoice approach responses to the pellets did not differ between the rats with vehicle infused or NMDA lesioned basolateral amygdalae (data not shown). Although the object approach response distributions are different in the BLA lesioned and intact rats, both groups demonstrate a trend in behaviorally recognizing the objects as positive or negative across time ($F(2,81)=365.34$, $p<0.0001$; see Figure 1 and Figure 2).

The microchoice data for the positive objects (those paired with the 100% sucrose and the 25% sucrose pellets) show that both the BLA lesioned and the vehicle infused rats have a "go" response nearly 100% of the time for all the time blocks examined (Figure 1A,B,C and Figure 2A,B,C). However, this rate of approach decreases for the microchoice data for the negative objects (those paired with the 0.002% and 0.02% Quinine HCl) at the beginning of the 1st time block. This decrease in the number of approaches to negatively associated objects is much more pronounced for the vehicle infused rats than for the BLA lesioned rats ($F(1,9)=104.81$, $p<0.0001$), although the change in the number of approaches to negative objects is significant for each group (vehicle: $F(3,23)=7.83$, $p<0.0001$; see Figure 1A; lesion: $F(3,19)=4.21$, $p=0.006$; see Figure 2A). By the end of training, both the vehicle infused and the BLA lesioned rats demonstrate decreased approach to negative objects (56% to 57% rate of approach for the BLA lesioned rats and 40% to 46% rate of approach for the vehicle infused rats; see Figure 1C and 2C).

Since the default response to visual objects was the "go", or approach response for all rats tested, subsequent analyses were completed separately for the "no go" responses to the negative objects. In order to focus the analysis on the differential approach behavior of the lesioned and the intact rats to the negative objects, step down ANOVAs were used to analyze the early and late stages of training separately for each group of animals (see Figure 3).

In the earliest stage of training, during the first half of time block one, the vehicle infused rats appropriately demonstrated less approach (fewer "go" responses) to the most aversive object ($F(1,23)=5.56$, $p=0.019$; see Figure 3A), while the BLA lesioned rats demonstrated equivalent degrees of approach to both negative objects ($F(1,19)=0.557$, $p=0.455$; see Figure 3A). In the subsequent stage of training, the second half of the first training block, both the vehicle infused ($F(1,23)=0.85$, $p=0.357$) and the BLA lesioned ($F(1,19)=1.61$, $p=0.21$) rats had equivalent levels of approach to negatively paired objects (see Figure 3B). By the last day of training, time block 4, the vehicle infused rats again demonstrated an appropriately decreased level of approach to the object associated with the worst food value ($F(1,23)=6.24$, $p=0.013$; see Figure 3C), while the BLA lesioned rats did not learn to distinguish between the negatively paired objects ($F(1,19)=0.006$, $p=0.936$; see Figure 3C). All of the microchoice approach data that yielded a "go" result over the first and the last training blocks for all animals was further analyzed for the time it took the rats to reach each of the objects. These data analyses are described below, in the "Latency Data" section of results.

4.2 Latency Data

In the analysis of latency data in this study, it was found that the rats with intact basolateral amygdalae demonstrated a significantly different distribution of latency responses toward the objects when compared with the BLA lesioned animals ($F(1,9)=13.27$, $p=0.005$; see Figure 4). Although the object approach response distributions were different in the BLA lesioned and intact rats, both groups demonstrated a trend in behaviorally recognizing the objects as positive or negative across time ($F(2,87)=22.47$, $p<0.0001$; see Figure 4). There was a significant interaction between the objects and the training blocks($F(6,87)=20.05$, $p<0.0001$), that served as evidence that both the BLA lesioned and the vehicle infused rat groups had different rates of learning objects based on affective value over time.

The significance of main effects allowed for the individual step down analyses of the time blocks for each group. One of the most striking results was that for each of the training time segments tested, the vehicle infused rats were consistently slower to reach all four different objects than the BLA lesioned rats (1^{st} Half Block 1: $F(1,9)=12.73$, $p=0.006$; 2^{nd} Half Block 1: $F(1,9)=13.37$, $p=0.005$; Block 4: $F(1,9)=16.62$, $p=0.003$; see Figure 4A, 4B, 4C respectively).

4.2.1 Post hoc comparisons: 1^{st} Half of Time Block 1

Post hoc comparisons of the performance of each of the two experimental groups on the latency to approach the different objects demonstrated that the vehicle infused rats learned to differentiate their instrumental responses between the affective value paired objects as early as the first half of the first training block ($F(3,23)=3.85$, $p=0.009$; see Figure 4A). Furthermore, the vehicle infused rats took significantly longer to approach

the two objects associated with intermediate values on the affective valence scale ("Good" and "Bad") than the two objects associated with the extreme values on the valence scale ("Best" and "Worst") ($F(1,23)=9.69$, $p=0.002$; see Figure 4A). Additionally, this early training time segment is the only one when the vehicle infused rats approached the "Best" object faster than the "Good" object, effectively demonstrating a differential instrumental response between two positive value associated objects ($F(1,23)=7.84$, $p=0.005$; see Figure 4A).

Conversely, the BLA lesioned rats approached all objects equally fast during the 1st half of the first training block ($F(3,19)=1.54$, $p=0.202$; see Figure 4A).

4.2.2 Post hoc comparisons: 2nd Half of Time Block 1

The vehicle infused rats continued to differentiate their instrumental responses according to the affective values associated with the objects during the second half of the first training block ($F(3,23)=2.73$, $p=0.043$; see Figure 4B). During this time segment, a distinct latency difference emerged between the positive and the negative value associated objects ($F(1,23)=7.36$, $p=0.007$; see Figure 4B). There were no latency differences between approaching the two positively associated objects ("Best" compared to "Good": $F(1,23)=0.21$, $p=0.65$; see Figure 4B) or between approaching the two negative value associated objects ("Worst" compared to "Bad": $F(1,23)=0.67$, $p=0.41$; see Figure 4B). In the BLA lesioned rat group, the rats approached all objects equally fast during the second half of the first training block ($F(3,19)=1.54$, $p=0.2$; see Figure 4B).

4.2.3 Post hoc comparisons: Time Block 4

By the final training block, time block 4, both the vehicle infused rats ($F(3,23)=40.96$, $p<0.0001$) and the BLA lesioned rats ($F(3,19)=37.73$, $p<0.0001$) differentiate their instrumental responses according to the affective values associated with the objects (see Figure 4C). During time block 4, the latency gap between the positive and the negative value associated objects widened for the vehicle infused rats ($F(1,23)=121.69$, $p<0.0001$; see Figure 4C). The same latency gap between the positive and the negative value associated objects was observed in the BLA lesioned animals ($F(1,19)=83.69$, $p<0.0001$; see Figure 4C). There were no latency differences between approaching the two positively associated objects ("Best" compared to "Good") for the vehicle infused rats ($F(1,23)=0.047$, $p=0.83$; see Figure 4C), as well as for the BLA lesioned animals ($F(1,19)=0.016$, $p=0.90$; see Figure 4C). Markedly, there were no latency differences between approaching the two negative value associated objects ("Worst" compared to "Bad") for the vehicle infused group of rats during the last time block ($F(1,23)=3.1$, $p=0.079$; see Figure 4C). However, the BLA lesioned rats approached the object paired with the most aversive food pellet (0.02% Quinine HCl) significantly slower than the object paired with the mildly aversive food pellet (0.002% Quinine HCl) ($F(1,19)=18.03$, $p<0.0001$; see Figure 4C).

5. Discussion

5.1 Microchoices: Approach and Avoidance.

The results of this experiment indicate that the basolateral amygdala bears significant influence on the emergence of instrumental responses to the objects associated with food reinforcements of gradated positive and negative affective values.

Furthermore, the BLA may be differentially involved in separate types of instrumental responses measured in this task. With respect to the microchoice responses, the vehicle infused animals in this study avoided the negatively associated objects more, compared to the BLA lesioned rats, who tended to approach negatively associated objects more often. With respect to approach latencies, the vehicle infused rats began discriminating between the objects according to associated values much earlier, and also consistently took a longer time to approach the objects than the BLA lesioned animals.

At the beginning of training on each set of objects, the rats were exposed to novel visual objects for the first time. The default response for both groups of rats was to approach the objects, eliciting the "go" response. The approach response had to be reversed for the negatively paired objects, as the rats learned the association between the objects and the different food values on the gradated valence continuum. The rats with an intact BLA were more successful at reversing their approach response to the bad objects than the BLA lesioned rats (see Figure 3A, 3B, 3C). This implies that the BLA lesioned animals were impaired at learning stimulus devaluation in an instrumental paradigm. This is consistent with the known effects of the BLA in stimulus devaluation and reversal of responses, described in many research studies with both animals and humans (Malkova et al., 1997; Baxter et al., 2000; Baxter and Murray, 2000; Hatfield et al., 1996; Gottfried et al, 2003). The evidence presented in Baxter et al. suggests that communication between the amygdala and the orbitofrontal cortex is necessary for adjusting the instrumental responses according to the change in the expected outcomes (Baxter et al., 2000; Baxter and Murray, 2000). The results from the current study are consistent with this hypothesis.

The vehicle infused rats demonstrated a very early distinction between the no-go responses to the two aversive objects. The consistent pairing with the bitter pellets seems to bias the vehicle infused rats to no-go to the "Bad" and to the "Worst" objects significantly more than the BLA lesioned animals. Early in training, specifically during the first half of time block 1, the vehicle infused rats distinguish the "Worst" object by avoiding it the most, an effect that is absent from the behavior of the BLA lesioned rats. This early distinction between the negative objects that is spared in the vehicle infused animals, is consistent with several different theories on the involvement of the BLA in instrumental learning (Killcross et al., 1998; Schoenbaum et al., 1999; Chiba et al., 2002). In the Killcross study, the authors discuss the disproportionate importance of the basolateral, compared to central, amydala in changing response patterns during aversive instrumental conditioning. In addition, single unit firing data shows that neurons in the BLA acquire selective firing properties to stimuli associated with aversive outcomes very early in training (Schoenbaum et al., 1999; Chiba et al., 2002).

The changes in the microchoice responses to negatively associated objects seem to be partially driven by the incentive value of the pellets paired with these objects. The incentive value has been construed in the literature to have both motivational (intensity) and sensory (valence) components (Balleine et al., 1997; Balleine, 2005; Corbit and Balleine, 2005). The pattern of changes in the microchoice responses of the vehicle infused rats is in line with a number of studies that explore the relationship between motivational values of stimuli, sensory properties of these stimuli, and the role of the BLA in instrumental learning (Balleine et al., 1997; Balleine et al., 2003; Blundell et al., 2003; Corbit and Balleine, 2005; Balleine, 2005).

The current findings on the differential approach to negative stimuli of different values support the Balleine hypothesis that the BLA participates in the selective sensitivity to the incentive value of the stimuli during instrumental responding (Corbit and Balleine, 2005; Balleine, 2005). However, the microchoice results are also consistent with the hypothesis that the motivational, or intensity, value and the valence gradations of the sensory properties of the stimuli are inextricably linked, albeit not in a linear, but in an interdependent manner (Winston et al., 2005; Balleine, 2005). A human fMRI study by Winston et al., demonstrated the involvement of the amygdala in this intensity-by-valence interaction in olfactory processing of affectively significant stimuli (Winston et al., 2005). The fact that the BLA lesioned rats show a reduced, but nevertheless significant reversal of approach response to the negatively associated objects, is also in direct support for the Corbit and Balleine hypothesis which postulates a distinct role for the central nucleus of the amygdala in motivated response learning.

Motor action planning and execution is inherent in the microchoice response to the objects, and has many elements of motivated motor learning, implicating the involvement of the connectivity between the basolateral amygdala and the striatal basal ganglia pathways (Everitt and Robbins, 1992, Alheid and Heimer, 1988, Parkinson et al., 2000). There is an established theory in literature that the BLA is important for early instrumental learning and the elicitation of appropriate behavioral responses to motivationally significant stimuli (Everitt and Robbins, 1992; Parkinson et al., 2000; Corbit and Balleine, 2005; Balleine, 2005). The findings on the approach/avoid behavior particularities of the BLA lesioned animals support the notion that the motivational

significance of the visual objects is no longer processed normally by the ventral striatum and the basal ganglia circuitry in these rats.

The overwhelming "Go" response to all objects displayed by the BLA lesioned animals through the last time block of training (see Figure 2C) help emphasize the importance of the interaction between the basolateral amygdala and the striatopallidal pathways which is necessary for the learning and elicitation of appropriate instrumental responses to learned stimuli (Everitt and Robbins, 1992; Parkinson et al., 2000; Corbit and Balleine, 2005; Balleine, 2005; Killcross et al., 1998). Although the vehicle infused rats also demonstrate a "Go" response to negative objects during the last training block their rate of approach is at least 10% to 15% less than that of the BLA lesioned animals (see Figure 1C).

The impulsivity of the microchoice behavior exhibited by the BLA lesioned rats, was evident in both the faster approach to all objects during training, and in the higher rate of object approach to negative objects (see Figure 2 and Figure 3). The absence of interconnectivity between the basolateral amygdala and the ventral striatal / basal ganglia circuitry potentially changed the way in which these structures normally influence incentive learning. This aberrant influence of the ventral striatal and striatopallidal circuits on the BLA lesioned rats' instrumental response patterns could explain the automaticity and impulsivity in their behavior (Everitt et al., 1989; Everitt et al., 2000; Everitt et al., 2001; Parkinson et al., 2000).

5.2 Approach Latencies

5.2.1 Summary of results

The results of this experiment show that the basolateral amygdala significantly contributes to the response distribution of approach latencies to objects associated with different affective values over time. The BLA is particularly important for the early formation of affective associations, as evident from the latency distribution in vehicle infused compared to the BLA lesioned rats in the first half of the first training block. For the intact rats, the latencies to reach the different value associated objects during the first half of the first training block visually resemble an inverse U-shaped learning curve (see Figure 4A). The behavior of the intact rats on latency responses during this early time segment shows that the rats can already distinguish some of the affective associabilities for these objects, if not yet able to direct their responses accordingly (see Figure 4A). This differential pattern of approach latencies to the affectively associated objects could be due to the interaction of the valence and arousal values of the pellets and their influence on visual processing (Vuilleumier et al., 2005; Winston et al., 2005). The broad implications of this hypothesis are discussed in detail below.

During the same early training time segment, the BLA lesioned animals show no changes in latencies with respect to object – valence associations, but they run to the objects significantly faster, taking less time to explore the objects (see Figure 4A).

The latency distributions are also strikingly different between the intact and the BLA lesioned animals in the 2nd half of the first training block. During this block, the intact rats ran much faster to the positive value associated objects than to the negative value associated objects (see Figure 4B), a very different pattern of instrumental responses compared to the first half of this training block. This pattern of response signals that the intact rats, even at this early time in training, can already associate the

novel objects with appropriate affective values. The approach latency distribution for the intact rats during the second half of the first training block was in accordance with the sensory and the motivational properties of the stimuli on the valence continuum (see Figure 4B).

Still, even with the faster running times to the positive objects, the intact rats took longer to approach all of the objects compared to the BLA lesioned animals, who again showed no change in any of the latencies in this time segment (see Figure 4B).

5.2.2 The role of the BLA in early associative learning

Thus, the BLA lesioned animals do not show any early learning on the latency of object approach, as evidenced by equivalent latencies of approach to all objects throughout the first time block of training. This is concrete evidence in support of the crucial role that the basolateral amygdala plays early in the associative learning process (Baxter et al., 2000; Baxter and Murray, 2000; Schoenbaum et al., 1999; Chiba et al., 2002).

However, from the distribution of the approach latencies during the last time block of training, it is evident that both the vehicle infused and the BLA lesioned rats are able to differentiate between objects according to the values of associated food reinforcements (see Figure 4C). During the last training time block the lesioned rats run faster not only to two positively associated objects relative to the two negatively associated objects, but they also run faster to the "Bad" object compared to the slowest latency "Worst" object (see Figure 4C).

The behavioral shift of the change in latencies to a later time point is also highly consistent with the late emergence of instrumental learning in the BLA lesioned rats compared to the vehicle infused rats. A number of structures, including the OFC, the central nucleus of the amygdala and the basal ganglia circuitry are potential substrate candidates for this redundant affective learning system (Killcross et al., 1998; Schoenbaum et al., 1998; Schoenbaum et al., 1999; Everitt and Robbins, 1992; Everitt et al., 2000).

Additionally, the fact that the BLA lesioned animals end up acquiring distinct responses to objects paired with different affective values may also be due to the presence of a stimulus - response component in the task, which is partially subserved by the striatal circuitry. It has been shown that although rats and monkeys with amygdala lesions are initially impaired at forming object – food associations, with extensive overtraining the animals may acquire these associations through sensorimotor learning (Malkova et al., 1997; Baxter et al., 2000; Baxter and Murray, 2002).

5.2.3 The role of the BLA in modulation of visual processing

Another aspect of the approach latency data revealed by the response differences between the intact and the lesioned rats is sufficiently important to warrant a discussion point. This is the prevalent tendency, throughout training, of the vehicle infused rats to take a longer time to run to and explore the objects longer before pushing them over. This significant difference in exploration time could be partially driven by the interaction between the amygdalar activity and the perceptual processing of the stimuli at the level of the visual cortex (Freese and Amaral, 2005; Adolphs, 2004; Vuilleumier et al., 2005).

The implication is that the BLA may be biasing exploration of relevant visual stimuli by altering visual sensory processing through the projections back to early visual cortical areas (Adolphs, 2004; Freese and Amaral, 2005).

Primary visual cortex is specialized for detection of distinct boundaries and contrasts in the visual fields. Through the process of lateral inhibition, neurons processing visual stimili that are similar in color, contrast, and orientation receive more inhibition, while neurons processing visual stimuli that are distinct in orientation, color, and contrast receive less inhibition, and thus more processing. Models of V1 that take into account most of the neural cortical attributes demonstrate natural enhancement of processing for the salient visual stimuli, also evident in visual search tasks, where it takes the model less time to find a dissimilar visual target among more homogeneous visual background (Li, 2003).

Such models of machine learning often use properties of cortical dynamics in the absence of modulation by the remote subcortical system. This is a very fundamental question of relevance in visual learning since this property is not modulated by cortex alone. There is evidence from both rats and monkeys that the basolateral amygdala sends projections directly to the earliest sensory processing areas of the visual cortex, namely V1 and V2 (Amaral et al., 2003; Freese and Amaral, 2005). The approach latency results discussed here are fully consistent with the hypothesis that the virtue of having a subcortical fast learning system, the basolateral amygdala, projecting directly to primary visual cortex is to bias visual processing to relevant stimuli in the environment (Adolphs, 2004).

The basolateral amydala could be selectively tuning the early visual areas to respond more strongly to salient stimuli, such as affectively associated visual objects. Although at the beginning of the first training block all rats encounter a novel set of visual objects, previous salient experience with objects could lead the vehicle infused rats to spend more time processing any visual object in the environment.

The longer running/exploration time of salient objects demonstrated by the vehicle infused rats in the current study could be interpreted several different ways based on this hypothesis. First, the selective BLA tuning of visual sensory areas in the intact rats could result in more processing for salient objects and lead to more "looking/exploring time" instead of pushing it over right away. Alternatively, without selective tuning, the visual cortex of the BLA lesioned animals could be processing all objects equivalently and suboptimally, resulting in poor visual recognition of affectively salient stimuli.

Ralph Adolphs wrote a brief review on the subject of emotional modulation of visual perception in humans (Adolphs, 2004). This paper provides support for the idea that emotional significance modulates visual regions of the brain through the amygdala (Amaral et al., 2003; Vuilleumier et al., 2005; Freese and Amaral, 2005). The review is concluded by some speculation as to the implications of recent evidence: do some people quite literally see things differently and do we have any control over our perception of visual stimuli? The results from the current experiment seem to provide support for Adolph's views.

This line of reasoning is also supported by the findings in a series of human fMRI studies linking the activation in the amygdala regions to a subsequent boost in visual cortex activation, and direction of visual resources to salient stimuli, such as fearful faces

(Adolphs et al., 2005; Adolphs, 2004; Vuilleumier et al., 2005; Ishai et al., 2002; Ishai et al., 2004; Ueda et al., 2003). This hypothesis is also consistent with human research on the "race effect" – the prevalent inability of people to visually differentiate faces of alternate racial origins – where amygdalar activation has also been implicated (Phelps et al., 2000; Hart et al., 2000).

In support of this interpretation of the latency data, there is also evidence from both human and animal studies that the amygdala receives visual information independently from subcortical pathways, such as the inputs from the superior colliculus and the thalamic visual inputs (Adolphs, 2004; Davis, 1993; Pegna et al., 2005).

5.3 Integration and Conclusion..

The vehicle infused animals alter both the speed and approach responses to objects as early as the first half of the first trianing block, according to the associated value of the food pellets paired with these objects. The approach response distributions seem to influence the changes in latencies for the vehicle infused animals. The BLA lesioned animals show no change in latencies to any of the objects for the duration of time block 1, while the microchoices during the same time change significantly to start avoiding the negatively associated objects. However, the changes in approach latency responses seem to be largely independent from the approach responses in the BLA lesioned animals.

The results show that while vehicle infused rats approach the "Worst" object significantly less than the "Bad" object on the last day of training, during the same time there is no difference between the approach latencies to these objects. This is another

important outcome of the relationship between the microchoice and latency response distributions – the relative values of the reinforcements potentially change if the animals encounter these reinforcements in variable quantities (Baxter and Murray, 2000; Kesner and Williams, 1995). Since the vehicle infused rats are overexposed to the less aversive pellet, it might become more, or just as aversive as the "Worst" pellet, as measured by the latency of approach in instrumental learning. This result is also strongly consistent with the hypothesis that the approach responses are partially driving the changes in approach latencies displayed by the vehicle infused rats.

The intact rats respond differently to the two negative objects with regard to the microchoice behavior but still run equally fast to the two negative objects. However, the BLA lesioned animals do not show the same kind of interaction between the microchoice responses and the approach latencies on the last block of training. Instead, the BLA lesioned animals approach both the "Bad" and the "Worst" object equally often, but run faster to the more aversive, "Worst" object. This distinction in latency of approach between two negatively associated objects could serve as further evidence that without the basolateral amygdala, other structures, such as the orbitofrontal cortex, the central nucleus of the amygdala, or the basal gangila circuitry are contributing to changes in approach latencies to affectively significant objects (Killcross et al., 1998; Baxter et al., 2002; Everitt and Robbins, 1992; Everitt et al., 2000).

On the other hand, the approach responding during late training is still more prevalent in the BLA lesioned animals, indicating that these late learning systems are not functioning normally in the absense of the basolateral amygala. This evidence is in support of the great degree of interaction between the BLA and the basal ganglia/ventral

striatal systems that has been discussed in literature (Everitt et al., 1991; Everitt and Robbins, 1992).

In conclusion, the basolateral amygdala seems to be a rapid associator that recruits and informs a number of other neural systems during learning of affectively significant stimuli. Redundancy of responding at different time scales is well suited to adaptive behavior. Without the amygdala, however, it would be difficult for an animal to adapt to a rapidly changing environment.

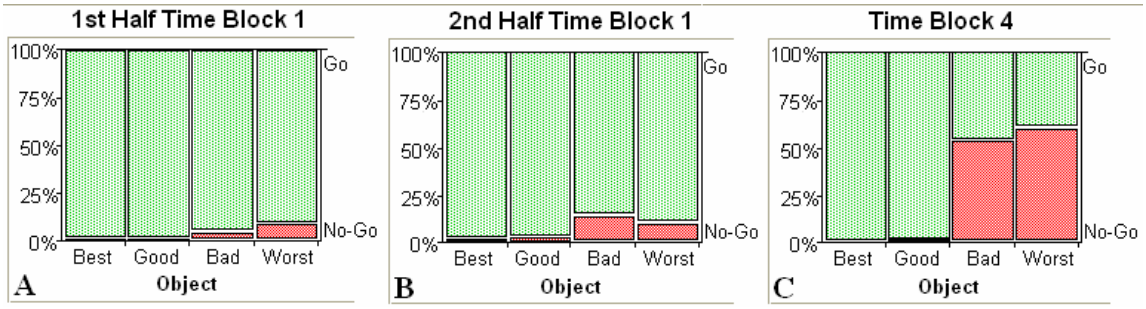


Figure 3.1 Object Approach: Vehicle Infused Rats

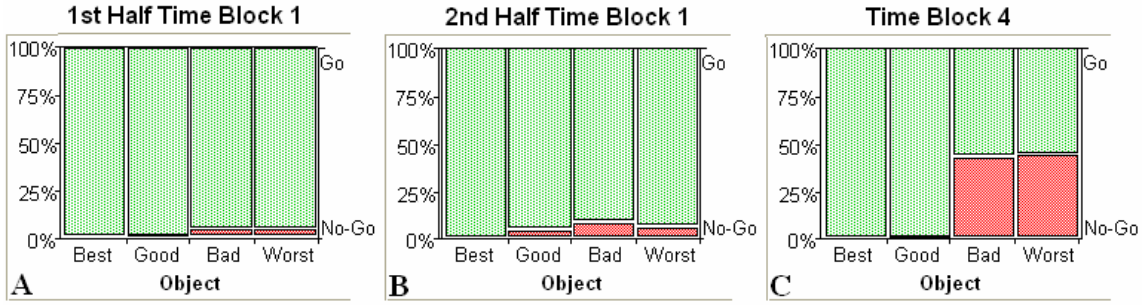


Figure 3.2 Object Approach: BLA Lesioned Rats

Figure 3.1 and 3.2 Behavioral approach – "go" and avoid – "no-go" responses to objects across time blocks of training by vehicle BLA infused (Figure 1) and NMDA in BLA infused (Figure 2) animals. Microchoice data was massed over trials and represented as percentage distribution of go and no-go responses (Y-axis) for each object (X-axis; "Best" paired with 100% sucrose, "Good" with 25% sucrose, "Bad" with 0.002% quinine, and "Worst" with 0.02% quinine). Each masaic plot represents massing of data from 6 vehicle infused **(1A-C)** and 5 NMDA infused **(2A-C)** animals over 4 to 8 different object sets for each animal. **(A)** Go – no-go responses each object from the 1st half of the 1st block of training, **(B)** Same for the 2nd half of the 1st block of training, **(C)** Same for the 4th (last) block of training.Each daily session began with the delivery of one free sugar pellet into the reward tray (bottom-left) and continued for 100 experimental trials. The release of the flap door, following either a reward or an error, triggered the next stimulus after a 5 sec intertrial interval (ITI). A timely response into a briefly illuminated port was rewarded with a sucrose pellet (a correct response) and an incorrect response turned off the house lights for 5 sec (a time out). Incorrect responses include responding before the presentation of a stimulus (a premature response), not responding to the stimulus within 3 sec period (an omission error), responding into a port that was not illuminated (incorrect response) and responding repeatedly into a port (a perseverative error, not shown). Credit: Cambridge Cognition.

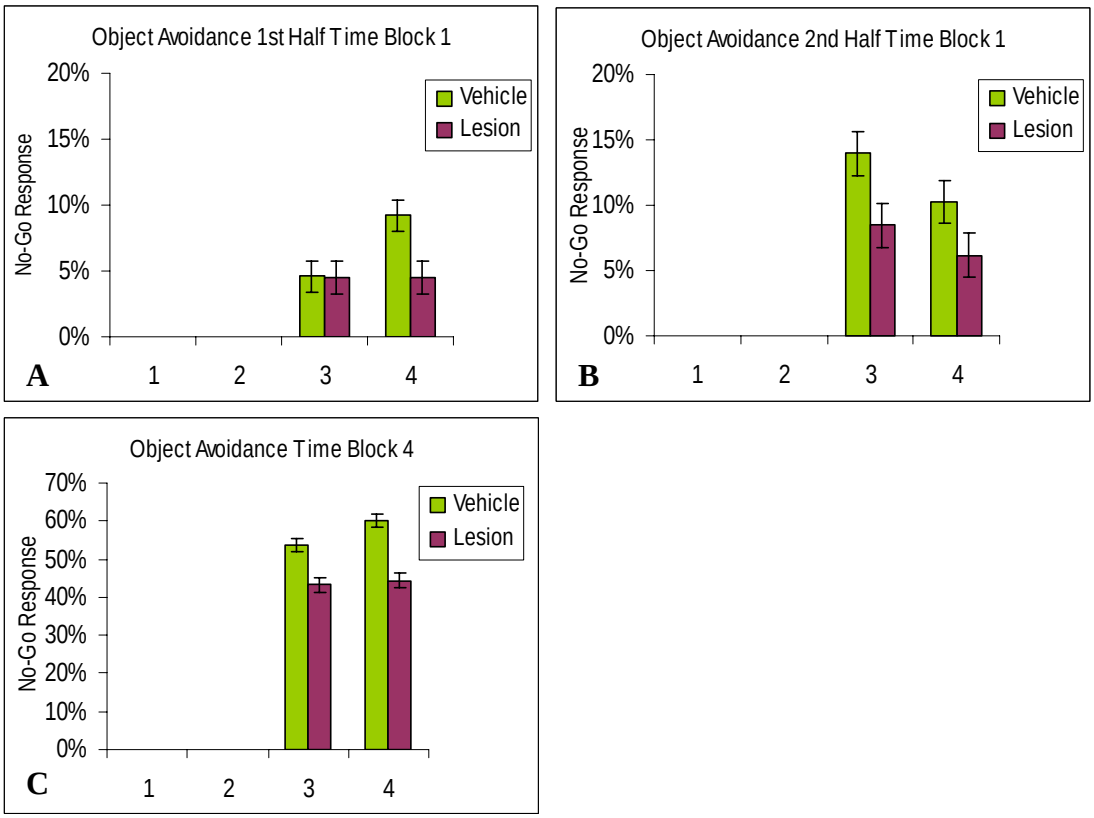


Figure 3.3 Behavioral avoidance – "no-go" responses to negative objects across time blocks of training for vehicle infused and BLA lesioned infused rats. The NoGo avoidance data was massed over trials and represented as percentage (Y-axis) of total go and no-go responses for each object (X-axis; "Best"(1) and "Good"(2) omitted, "Bad"(3) paired with 0.002% quinine, and "Worst"(4) paired 0.02% quinine). Each plot represents massing of data from 6 vehicle infused and 5 NMDA infused animals over 4 to 8 different object sets for each animal. **(A)** NoGo responses for negative objects from the 1st half of the 1st block of training, **(B)** Same for the 2nd half of the 1st block of training, **(C)** Same for the 4th (last) block of training.

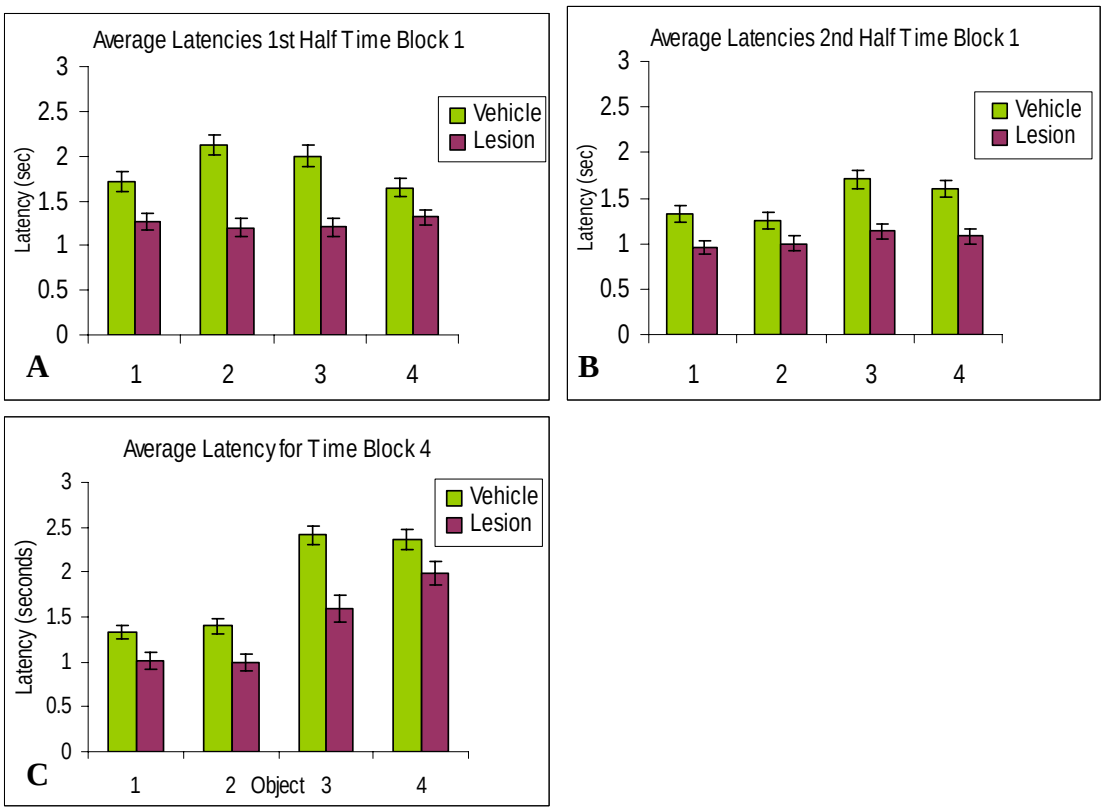


Figure 3.4 Latency of approach to objects for the 1st and the last time block of object exposure in the BLA lesioned (5 animals) and vehicle (6 animals) infused rats. The approach latency responses were pooled and averaged for each group. The average latencies in seconds (Y-axis) were plotted by all objects for each group (X-axis; "Best"(1) paired with 100% sucrose, "Good" with 25% sucrose, "Bad"(3) paired with 0.002% quinine, and "Worst"(4) paired 0.02% quinine). **(A)** In the 1st half of time block 1, control animals show longer approach latencies to novel objects relative to lesioned animals, as well as a distinct pattern of approach latencies to each object. **(B)** In the 2nd half of time block 1, control animals show a distinction between the positively and the negatively associated objects, while the lesion animals demonstrate latencies similar to (A). **(C)** In the 4th time block, control animals show differential approach latencies to good and bad objects, and longer approach latencies relative to lesioned animals who also show a distinct pattern of approach latencies to different objects.

6. References

Adolphs R, Gosselin F, Buchanan TW, Tranel D, Schyns P, Damasio AR. A mechanism for impaired fear recognition after amygdala damage. Nature. 2005 Jan 6;433(7021):68-72.

Adolphs, Ralph, Emotional Vision, Nat. Neurosci. 2004 Nov; 7(11):1167-8.

Alheid, G. F., & Heimer, L. (1988). New perspectives in basal forebrain organization of special relevance for neuropsychiatric disorders: the striatopallidal, amygdaloid, and corticopetal components of substantia innominata. Neuroscience, 27(1), 1-39.

Amaral DG, Behniea H, Kelly JL. Topographic organization of projections from the amygdala to the visual cortex in the macaque monkey. Neuroscience. 2003;118(4):1099-120.

Balleine BW. Neural bases of food-seeking: Affect, arousal and reward in corticostriatolimbic circuits. Physiol Behav. 2005 Dec 15;86(5):717-30. Epub 2005 Oct 27

Balleine, B. W., Killcross, A. S., & Dickinson, A. (2003). The effect of lesions of the basolateral amygdala on instrumental conditioning. J Neurosci, 23(2), 666-675.

Balleine, B.W., Leibeskind, J.C. and Dickinson, A.: Effects of cell body lesions of the Basolateral Amygdala on instrumental conditioning. Soc. Neuroscience Abstract 1997, 23, 786.

Blundell P, Hall G, Killcross S. Preserved sensitivity to outcome value after lesions of the basolateral amygdala. J Neurosci. 2003 Aug 20;23(20):7702-9.

Chiba A.A., Quinn L..K., Merzlyak I.Y. (2002). Neural activity in the rat basolateral amygdala reflects the acquired motivational significance of visual objects. Program No. 284.8. 2002 Abstract Viewer/Itinerary Planner. Washington, DC: Society for Neuroscience, 2002.

Corbit LH, Balleine BW. Double dissociation of basolateral and central amygdala lesions on the general and outcome-specific forms of pavlovian-instrumental transfer. J Neurosci. 2005 Jan 26;25(4):962-70.

Davis M. Pharmacological analysis of fear-potentiated startle. Braz J Med Biol Res. 1993 Mar;26(3):235-60.

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., & Whalen, P. J. (2001). The amygdala: vigilance and emotion. Mol Psychiatry, 6(1), 13-34.

Dringenberg HC, Kuo MC, Tomaszek S., Stabilization of thalamo-cortical long-term potentiation by the amygdala: cholinergic and transcription-dependent mechanisms, Eur J Neurosci. 2004 Jul;20(2):557-65.

Everitt BJ, Cardinal RN, Hall J, Parkinson J, Robbins T. (2000). Differential involvement of amygdala subsystems in appetitve conditioning and drug addiction. In The Amygdala. Edited by JP Aggleton. New York City: Oxford University Press; 353-381.

Everitt, B. J., Cador, M., & Robbins, T. W. (1989). Interactions between the amygdala and ventral striatum in stimulus-reward associations: studies using a second-order schedule of sexual reinforcement. Neuroscience, 30(1), 63-75.

Everitt, B. J., Dickinson, A., & Robbins, T. W. (2001). The neuropsychological basis of addictive behaviour. Brain Res Brain Res Rev, 36(2-3), 129-138.

Everitt, B. J., Morris, K. A., O'Brien, A., & Robbins, T. W. (1991). The basolateral amygdala-ventral striatal system and conditioned place preference: further evidence of limbic-striatal interactions underlying reward-related processes. Neuroscience, 42(1), 1-18.

Everitt, B.J., Robbins, T,W. (1992). Amygdala-ventral striatal interactions and reward-related processes. In The Amygdala. Edited by JP Aggleton. New York City: Wiley-Liss; 401-430.

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. Behav Neurosci, 108(1), 210-212.

Freese JL, Amaral DG., The organization of projections from the amygdala to visual cortical areas TE and V1 in the macaque monkey, J Comp Neurol. 2005 Jun 13;486(4):295-317.

Gallagher, M., & Chiba, A. A. (1996). The amygdala and emotion. Curr Opin Neurobiol, 6(2), 221-227.

Gottfried JA, O'Doherty J, Dolan RJ. Encoding predictive reward value in human amygdala and orbitofrontal cortex. Science. 2003 Aug 22;301(5636):1104-7.

Hart AJ, Whalen PJ, Shin LM, McInerney SC, Fischer H, Rauch SL. Differential response in the human amygdala to racial outgroup vs ingroup face stimuli. Neuroreport. 2000 Aug 3;11(11):2351-5

Hatfield, T., Han, J. S., Conley, M., Gallagher, M., & Holland, P. (1996). Neurotoxic lesions of basolateral, but not central, amygdala interfere with Pavlovian second-order conditioning and reinforcer devaluation effects. J Neurosci, 16(16), 5256-5265.

Heimer, L., Alheid, G. F., de Olmos, J. S., Groenewegen, H. J., Haber, S. N., Harlan, R. E., & Zahm, D. S. (1997). The accumbens: beyond the core-shell dichotomy. J Neuropsychiatry Clin Neurosci, 9(3), 354-381.

Hiroi, N., & White, N. M. (1991). The lateral nucleus of the amygdala mediates expression of the amphetamine-produced conditioned place preference. J Neurosci, 11(7), 2107-2116.

Holland, P. C., Chik, Y., & Zhang, Q. (2001). Inhibitory learning tests of conditioned stimulus associability in rats with lesions of the amygdala central nucleus. Behav Neurosci, 115(5), 1154-1158.

Ishai A, Haxby JV, Ungerleider LG. 2002b Visual imagery of famous faces: effects of memory and attention revealed by fMRI. Neuroimage. 2002 Dec;17(4):1729-41

Ishai A, Pessoa L, Bikle PC, Ungerleider LG., 2004 Repetition suppression of faces is modulated by emotion, Proc Natl Acad Sci U S A. 2004 Jun 29;101(26):9827-32. Epub 2004 Jun 21.

Killcross, S., Robbins, T. W., & Everitt, B. J. (1997). Different types of fear-conditioned behaviour mediated by separate nuclei within amygdala. Nature, 388(6640), 377-380.

LeDoux, J. E., Cicchetti, P., Xagoraris, A., & Romanski, L. M. (1990). The lateral amygdaloid nucleus: sensory interface of the amygdala in fear conditioning. J Neurosci, 10(4), 1062-1069.

LeDoux, J. E., Iwata, J., Pearl, D., & Reis, D. J. (1986). Disruption of auditory but not visual learning by destruction of intrinsic neurons in the rat medial geniculate body. Brain Res, 371(2), 395-399.

Liang, KC. And Chiang, TC. (1994) Locus coeruleus infusion of clonidine impaired retention and attenuated memory enhancing effects of epinephrine. Society for Neuroscience Abstracts, 20, 153.

Li, Z. (2002). A saliency map in primary visual cortex. Trends Cogn Sci., Jan 1;6(1):9-16.

Malkova, L., Gaffan, D., & Murray, E. A. (1997). Excitotoxic lesions of the amygdala fail to produce impairment in visual learning for auditory secondary reinforcement but interfere with reinforcer devaluation effects in rhesus monkeys. J Neurosci, 17(15), 6011-6020.

McDonald, A. J. (1998). Cortical pathways to the mammalian amygdala. Prog Neurobiol, 55(3), 257-332.

McDonald, A. J., & Mascagni, F. (1996). Cortico-cortical and cortico-amygdaloid projections of the rat occipital cortex: a Phaseolus vulgaris leucoagglutinin study. Neuroscience, 71(1), 37-54.

McGaugh, J. L., Cahill, L., & Roozendaal, B. (1996). Involvement of the amygdala in memory storage: interaction with other brain systems. Proc Natl Acad Sci U S A, 93(24), 13508-13514.

McGaugh, J.L., Introini-Collison ,I.B., Cahill, L., Kim, M., Liang, K.C.(1992). Involvement of the amygdala in neuromodulatory influences on memory storage. In The Amygdala. Edited by JP Aggleton. New York City: Wiley-Liss; (1992):431-452.

McGaugh, J.L., Ferry B, Vazdarjanova, A, Roozendaal, B. (2000). Amygdala: role in modulation of memory storage. In The Amygdala. Edited by JP Aggleton. New York City: Oxford University Press, 391-412.

Miyashita, T., & Williams, C. L. (2002). Glutamatergic transmission in the nucleus of the solitary tract modulates memory through influences on amygdala noradrenergic systems. Behav Neurosci, 116(1), 13-21.

Parkinson, J. A., Cardinal, R. N., & Everitt, B. J. (2000). Limbic cortical-ventral striatal systems underlying appetitive conditioning. Prog Brain Res, 126, 263-285.

Pegna AJ, Khateb A, Lazeyras F, Seghier ML., Discriminating emotional faces without primary visual cortices involves the right amygdala, Nat Neurosci. 2005 Jan;8(1):24-5. Epub 2004 Dec 12.

Phelps EA, O'Connor KJ, Cunningham WA, Funayama ES, Gatenby JC, Gore JC, Banaji MR. Performance on indirect measures of race evaluation predicts amygdala activation. J Cogn Neurosci. 2000 Sep;12(5):729-38

Pitkanen, A., Stefanacci, L., Farb, C. R., Go, G. G., LeDoux, J. E., & Amaral, D. G. (1995). Intrinsic connections of the rat amygdaloid complex: projections originating in the lateral nucleus. J Comp Neurol, 356(2), 288-310.

Price JL, Fokje TR, Amaral, DG. (1987) The limbic region. II: The amygdaloid complex. In Handbook of Chemical Neuroanatomy, edited by Bjorklund, Hokfelt, Swanson. Elsevier Science Publishers. 279-388.

Roozendaal, B., Williams, C. L., & McGaugh, J. L. (1999). Glucocorticoid receptor activation in the rat nucleus of the solitary tract facilitates memory consolidation: involvement of the basolateral amygdala. Eur J Neurosci, 11(4), 1317-1323.

Roozendaal, B. (2000). 1999 Curt P. Richter award. Glucocorticoids and the regulation of memory consolidation. Psychoneuroendocrinology, 25(3), 213-238.

Quinn L.K., Merzlyak I.Y., Minces V., Chiba A.A. Neural Activity in the Rat Basolateral Amygdala Reflects the Acquired Motivational Significance of Visual Objects (in preparation).

Salinas, J. A., & McGaugh, J. L. (1995). Muscimol induces retrograde amnesia for changes in reward magnitude. Neurobiol Learn Mem, 63(3), 277-285.

Salinas, J. A., Parent, M. B., & McGaugh, J. L. (1996). Ibotenic acid lesions of the amygdala basolateral complex or central nucleus differentially effect the response to reductions in reward. Brain Res, 742(1-2), 283-293.

Schoenbaum, G., Chiba, A. A., & Gallagher, M. (1999). Neural encoding in orbitofrontal cortex and basolateral amygdala during olfactory discrimination learning. J Neurosci, 19(5), 1876-1884.

Schoenbaum G, Chiba AA, Gallagher M. Orbitofrontal cortex and basolateral amygdala encode expected outcomes during learning. Nat Neurosci. 1998 Jun;1(2):155-9.

Schoenbaum G, Setlow B, Saddoris MP, Gallagher M. Encoding predicted outcome and acquired value in orbitofrontal cortex during cue sampling depends upon input from basolateral amygdala. Neuron. 2003 Aug 28;39(5):855-67.

Swanson, L. W., & Petrovich, G. D. (1998). What is the amygdala? Trends Neurosci, 21(8), 323-331

Tabbert K, Stark R, Kirsch P, Vaitl D., Hemodynamic responses of the amygdala, the orbitofrontal cortex and the visual cortex during a fear conditioning paradigm, Int J Psychophysiol. 2005 Jul;57(1):15-23. Epub 2005 Apr 21.

Ueda, K., Okamoto, Y., Okada, G., Yamashita, H., Hori, T., Yamawaki, S., (2003). Brain activity during expectancy of emotional stimuli: an fMRI study. Neuroreport 14, 51– 55.

Vuilleumier P, Richardson MP, Armony JL, Driver J, Dolan RJ, Distant influences of amygdala lesion on visual cortical activation during emotional face processing, Nat Neurosci. 2004 Nov;7(11):1271-8. Epub 2004 Oct 24.

Wang SH, Ostlund SB, Nader K, Balleine BW. Consolidation and reconsolidation of incentive learning in the amygdala. J Neurosci. 2005 Jan 26;25(4):830-5.

Winston JS, Gottfried JA, Kilner JM, Dolan RJ. Integrated neural representations of odor intensity and affective valence in human amygdala. J Neurosci. 2005 Sep 28;25(39):8903-7.

IV

Neural Mechanisms of Allostasis

1. Abstract

Allostasis and allostatic load have been discussed extensively in existing literature as phenomena pertaining to neural disregulation inherent in stress, drug addiction, and a variety of neural and behavioral disorders. This chapter aims not only to summarize and generalize the statements and claims made about allostatic processing, but also to incorporate findings on learning, memory, and cognition into the allostatic framework. Works on aversive as well as appetitive stress and dysregulation are reviewed, along with findings on the involvement of specific neurochemical systems. The neurochemical systems reviewed are implicated in both routine as well as allostatic behavioral, motivational, and cognitive processing. These findings are incorporated with what is known about the extent of neuroanatomical interconnectivity of the relevant structures, in order to present an integrated framework of the neural control of allostasis.

2. Introduction

Behavior can be viewed as a coherent stream of physiological output that is initiated and coordinated by the brain. Behavior can be observed, measured, and manipulated, and this makes behavior a very important source of information from which the physiological and psychological levels of function in the brain can be inferred. The physiological and psychological parameters in the brain are influenced and modulated by changes in the environment that serve as input or stimuli to the organism. Interpretation and appraisal of information from the environment takes place through cognitive

mechanisms that result in memory or experience of this information. Organismal level function is broadly coordinated across many different parameters to yeild cohesive behavior in response to a given situation or environment. Through incorporating experience, such as memory, the behavior is adjusted through feedback from both environmental and neural demands.

The theory of homeostasis is based on the existence of specific set-points for each physiological parameter of a given organ in an organism, where the function of this organ is at the optimal level for living. Homeostasis is essentially a need of the organism to reach this balanced state by adjusting the physiological parameters toward these set-points.

In homeostasis there is no assumed communication between different organs. Regulation occurs on a local level without preemptive or anticipatory change in the physiological parameters.

Alternatively, allostasis implies that physiological parameters in an organism do not necessarily need to remain within a specific range or always aim towards the set-points in order to maintain stable function at organismal level (Koob and Le Moal, 2001; Schulkin et al., 1994; Sterling and Eyer, 1988).

Allostasis was originally proposed by Sterling and Eyer as a hypothetical alternative to homeostasis in order to explain arousal pathology (Sterling and Eyer, 1988). Specifically, these authors applied the concept of allostasis to brain control of cardiovascular activity. Their idea was to explain the variability in blood pressure, for instance, as broadly coordinated and dependent on the environmental situations and internal states, both physiological and psychological.

Allostasis implies that physiology is adjusted according to demand, specifically through evaluation and anticipation of demands, as opposed to automatically returning to a pre-determined "set-point". Thus, allostasis is an alternative regulatory theory to homeostasis that takes environmental and internal neural stimuli into account to provide a coordinated response on all levels of function, including cognitive and behavioral. The feedback signals involved in neural control of allostasis are positive, as well as negative, to accordingly inform the brain of the continuous change in demand (McEwen, 2000; Koob and Le Moal, 2001). By virtue of the interconnectedness of the neural circuits that receive this feedback (Heimer et al., 1997), there is opportunity for anticipating the demand and, therefore, the planning of the physiological output is informed. Since past experience of external stimuli, such as learning and memory, is incorporated into this framework, allostasis represents a built-in mechanism for anticipatory change in psychology and physiology in accordance with demand.

As a result, the organism is in a state of functional stability that is acheived through continuous change in physiological parameters (Sterling and Eyer, 1988). The model of allostasis, compared with homeostasis, allows for a more accurate resolution of the brain-body relationship. The overarching hypothesis that is proposed here is an intimate connection between the brain-behavior and the brain-body relationship. In this hypothesis, cognitive appraisal of information and feedback to the brain about physiological arousal play a central part in neural control of organismal functions.

In cases where there is continuous demand, the organism maintains apparent functional stability only through the constantly changing physiology, yielding an allostatic, instead of a homeostatic state (Koob and LeMoal, 2001). However, persistent

departure from the balanced homeostatic state is detrimental to the body, and yields pathophysiological states that are no longer optimal for living (Schulkin et al., 1994; McEwen, 1998). This has been termed "allostatic load" -- the cost to the brain and body caused by extended activation of allostatic processes through extreme demands on the organism (Schulkin et al., 1994; McEwen, 1998).

Short term allostatic processing is beneficial for keeping an organism in a functional state (McEwen, 2000). A hypothesis set forth by McEwen is that allostatic load is a result of dysfunction in normal allostatic processing, where four different types of allostatic dysfunction are looked at: repetitive allostatic response in response to repetitive stress, lack of adaptation in the repetitive allostatic response, inadequate allostatic response to stress, and allostatic response to stress that is not shut off when the stress is aleviated (McEwen, 2000). All of these can be summarized as dysregulation in normal allostatic functioning.

According to McEwen, allostatic load can be assessed by looking at chronic dysregulation of physiological parameters where a non-stressed, functional state is assumed as baseline (2000). Deviation from this known baseline can be measured through assessments of physiology, such as levels of stress hormones and/or cardiovascular response (Seeman et al., 1997; Seeman et al., 2001). One of the most important factors in dysregulation of allostasis, with respect to placing demands on the organismal function, is stress (Sterling and Eyer, 1988; Schulkin et al., 1994; McEwen, 2000; McEwen, 2001).

3. Stress and Allostasis

In order to understand allostasis and allostatic load, it is vitally important to consider the involvement of stress in these processes. Stress engages allostatic processes, and chronic stress leads to allostatic load (McEwen et al., 1999; Koob and LeMoal, 2001). Allostasis was originally proposed as a paradigm to explain arousal pathology (Sterling and Eyer, 1988). Chronic stress leads to arousal pathology – where there is constant deviation from baseline physiological parameters. The neural systems involved in control of allostasis are also involved in regulating stress pathology (Koob and Le Moal, 2001; McEwen et al., 1999).

3.1 Definition of Stress

Stress can be defined from both physiological and psychological perspectives as a response of the organism to a perturbing stimulus. In the case of physiological, or, somatic types of stress, the stressful stimuli are noxious physiological demands upon the body (Selye, 1936). In the case of social or psychological stress, the stimuli are demands upon both the brain and the body that could be either internally or externally generated. (Burchfield, 1979) When subjected to a stressful stimulus, a stress response is initiated in an organism. For both environmental and internally generated stressors, the brain and/or body first interpret the stimulus as stressful, and then a cascade of physiological changes both in the brain and in the body occurs.

3.2 Stress Pathways

The interpretation of a stressful stimulus or situation takes place through sensory cortices passing information about the perceived stressor through the thalamic relay to the

hypothalamus, or, in addition, could also involve cognitive processing of the stressful stimulus including appraisal of the situation. In either case, the body's stress response involves the activation of the Hypothalamo Pituitary Adrenal (HPA) system in parallel to the behavioral response to stress which is aided by HPA activation (Marrocco et al., 1994). The hypothalamus is the effector structure in the brain that activates the neuroendocrine component of the response to stress through the HPA. The hypothalamus also participates in the activation of the behavioral component of the stress response through connections to both the Autonomic Nervous System (ANS) and to the extra-hypothalamic CNS stress circuits (Hugdahl, 1996; Koob and Le Moal, 2001). The extra-hypothalamic stress circuits include the amygdala, the bed nucleus of stria terminalis, and their associated structures (Koob and Le Moal, 2001).

3.3 Structures of Stress Response

The brain stress systems, also known as the extra-hypothalamic CRF stress systems, include the amygdala and the bed nucleus of stria terminalis (BNST) as the key CRF producing loci, which in turn project to many other cortical and subcortical structures all over the brain (Koob and Le Moal, 2001).

The behavioral component of the stress response is mediated in part by ANS activity and in part through the connections between three anatomical constructs, the Cortico-Striatal-Thalamic loop, as a motivated action generator (Graybiel et al., 1994), the amygdala, as a structure involved in appraisal of stressful information (Davis, 1994), and the brainstem motor nuclei.

3.4 Allostatic Load

Failure or prolonged activation of either neuroendocrine, autonomic, or behavioral stress response components may lead to a pathological state, or allostatic load, through the dysregulation of endocrine and other stress mediated neurochemical systems. Some examples of allostatic load at the level of organismal function are hypertension, cardiovascular disease, and generally suppressed immune function (Sterling and Eyer, 1988; McEwen, 2000a). All of these conditions are precipitated or exacerbated by chronic stress. There are also numerous mental disorders that can be precipitated or exacerbated by chronic stress, including post-traumatic stress disorder (PTSD), major depressive disorder (MDD), schizophrenia, and multiple other illnesses (Friedman, 1997; McEwen et al., 1997; Michelson and Gold, 1998).

An operationalized measure of allostatic load (AL) was constructed by T. Seeman and collegues, after completing several longitudinal studies which characterized the incidence of a dysregulation of a cohort of physiological parameters (Seeman et al., 1997; Seeman et al., 2001). The parameters included in the operationalized definition of AL included cardiovascular measures, measures of systemic steroids, such as epinephrine and cortisol, as well as several measures of constitution, such as waist to hip ratio, and cholesterol (Seeman et al., 2001).

Some instances where allostatic load effects were observed directly include research studies with injuries in elite athletes, biological risks in the elderly, early age at menarche, and incidence of depression in post traumatic brain injury patients (Galambos et al., 2005; Seeman et al., 2001; Allsworth et al., 2005; Bay et al., 2004). In the case of elite athletes, the findings showed that such allostatic load correlates as mood and

perceived stress could successfully account for some of the injury related vairables which the athletes were susceptible to (Galambos et al., 2005). Some crucial measures, however, such as hormonal and free cortisol levels, were absent from the analysis, but could potentially account for many more injury related variables (Galambos et al., 2005). Another study explored the relationship between early onset of menstrual function, which has been associated with a host of health risks, and biological measures of allostatic load, including cardiovascular, metabolic, and immune parameters, using the operationalized definition of AL (Allsworth et al., 2005; Seeman et al., 2001). The authors found a strong positive correlation between high allostatic load scores, and the early onset of menarche, controlling for such factors as race and socioeconomic levels (Allsworth et al., 2005). High allostatic load scores were also predictive of increased mortality, decreased cognitive functioning, and health risks in the elderly (Seeman et al., 2001). Chronic stress, as one of the indicators of allostatic load, also significantly explained the occurrence of depressive symptoms in patients who sustained traumatic brain injury (Bay et al., 2004).

There are some questions of causality between the different populations and parameters of AL that systematically vary in those populations. However, the high incidence of co-morbidity of the AL measures suggest that allostatic load is a valid and a measurable risk factor for numerous deseases and health factors (Seeman et al., 2001; Szanton et al., 2005; Hellhammer et al., 2004).

3.5 Disregulation of Neural Functions during Allostatic Load

As opposed to these examples of allostatic dysregulation on a broad organismal level, there are several instances of allostatic state resulting from dysregulation of specific neural functions that have been presented in the literature on allostasis and stress. These include effects of chronic stress on spatial memory, and stress mediated dysregulation of reward as it occurs during drug addiction (Luine et al., 1996; Koob and Le Moal, 1997). There are also several examples of acute stress influencing neural function. These instances include enhanced consolidation of emotionally significant memories during stress, and deletarious effects of stress on working memory (Birnbaum et al., 1999; Quirarte et al., 1997; Roozendaal, 1999; McGaugh et al., 1992). Acute stress triggers allostatic response in these cases, while chronic stress could potentially exacerbate this response to the level of allostatic load. The neural mechanisms for all of these examples of allostatic state and allostatic load will be discussed here in detail, as well as the modes through which they reached this degree of allostatic dysregulation.

4. Neural Substrates of Allostasis

4.1 Key Structures

The neural structures involved in all the functions described above are highly interconnected and receive inputs from neural circuits involved in the stress response. The hippocampus is involved in the effects of stress on spatial memory, the amygdala participates in the consolidation of affectively significant memories, and the involvement of the prefrontal cortex is important for working memory tasks (Birnbaum et al., 1999; Quirarte et al., 1997; Roozendaal, 1999; McGaugh et al., 1992; Luine et al., 1996; Koob and Le Moal, 1997). Several structures including the amygdala, nucleus accumbens, and

the Basal Ganglia (as an integral part of the Cortico-Striatal-Thalamic loop) are important for the mechanisms involved in drug addiction (Koob, 1992; Koob and Le Moal, 2001). The neural circuitry involved in allostatic processing shares important characteristics in addition to the interconnectivity patterns. The neurotransmitters released by the structures involved in allostasis determine the functional significance of this circuitry.

4.2 Key Neurotransmitter Systems

There are several key neurotransmitter and neuromodulatory systems that participate in allostatic processes and that are liable to become imbalanced in cases of allostatic dysregulation. These include CRF, synthesis and release of this neuromodulator is upregulated during allostatic load (Gray, 1993; Schulkin et al., 1998; McEwen and Sapolsky, 1995). Another neuromodulator, dopamine (DA), is broadly involved in mechanisms of reward and motivation (Heimer et al., 1997; Schultz, 1998). The imbalance of DA plays an important role in reward dysregulation involved in allostatic load that is precipitated by both behavioral and physiological aspects of drug addiction (Koob and Le Moal, 1997). During drug addiction, DA is downregulated in certain neural structures, with selective sensitization of receptors taking place as drug addiction progresses, as is especially evident in cortico-striatal-thalamic loop function (Koob and Le Moal, 2001; Graybiel et al., 1994; Abercrombie and Zigmond, 1995). Dopamine systems in the Prefrontal Cortex (PFC) play a crucial role in memory tasks, and are subject to disregulation by the glucocorticoids during chronic stress, which adversely affects cognition (Arnsten, 2000; Mizoguchi et al., 2002; Mizoguchi et al., 2004). The noradrenergic system also has a prominent role in allostatic dysregulation

(Koob, 1999). In general, NE is involved in CNS and PNS arousal mechanisms, involving both affective and physiological arousal (Marrocco et al., 1994). The noradrenergic system has also been implicated in orienting behavior and attentional processing in addition to a general function in arousal (Foote et al., 1991). NE is upregulated globally during a stress response, and fails to return to baseline during chronic stress (Koob, 1999).

4.3 Mediation of Arousal and Stress Response

Both NE and glucocorticoids act on many biological targets, including sites in the brain, where they signal the level of arousal and stress response in the body (Marrocco et al., 1994; Charney et al., 1995). Once CRF triggers glucocorticoid production through HPA activation, glucocorticoids in turn shut off CRF production through negative feedback to some CRF synthesizing sites in the brain, such as the hypothalamus (McEwen and Sapolsky, 1995; Schulkin et al., 1998). Crucially to the model of allostasis presented here, glucocorticoids trigger more CRF production in the amygdala and BNST through positive feedback to these structures (Koob and LeMoal, 2001). The amygdala and BNST synthesize more CRF in response to prolonged stressful stimuli and elicit further stress response in the CNS by recruiting the participation of the interconnected neural circuits that mediate the effects of allostasis and allostatic load (Gray, 1993; Koob and Le Moal, 2001).

4.4 Connection between Drug Addiction and Stress

It can be argued that the dysregulation of allostasis is a cumulative function of all the neural systems affected by stress. In fact, the neural circuits involved in stress are the same ones activated during drug addiction, and continue to be active during many drug withdrawal and dependence symptoms (Koob and LeMoal, 1997). Thus, drug addiction has been hypothesized to be one form of allostatic load (Koob and LeMoal, 2001). A neural connection and a functional parallel are implied between reward and stress mechanisms in the brain, and the consequences of their dysregulation.

"Too much of a good thing", as can happen during excessive reward mechanism activation preceeding drug addiction, and "too much of a bad thing", such as chronic stress, both have negative consequenses for maintaining physiological baselines and functional stability in an organism. The pathological state of allostatic load can be induced through either reward dysregulation, as occures in drug addiction, or dysregulation of the stress response mechanisms through chronic stress (Koob and LeMoal, 2001; Schulkin et al., 1994).

4.5 Allostatic Response can be Neuroprotective

In moderation, both reward and stress responses are highly useful and necessary for normal functioning of the organism, implicating regulated allostatic processing. Initiation of allostatic processing in response to stress is a form of a coping response, and as such it may serve as a neuroprotective factor (Sterling and Eyer, 1988; Koob and Le Moal, 1997; McEwen, 2000b). In the view presented in these papers, regulated allostasis is an adaptation to the environmental and neural demands, while dysregulated allostasis, or allostatic load, is an adaptation gone awry (McEwen, 2000b; Seeman et al., 2001).

Allostasis is a system wide phenomenon, based on the interconnectedness of all the neural systems involved. One of the implications that stems from this degree of neural communication is that allostatic pathology resulting from dysregulation of a particular neural system will also produce a vulnarability to other types of allostatic pathologies (Koob and Le Moal, 2001). Further, based on the involvement of such globally active neuromodulators as CRF, DA, and NE, there is a sizeable potential for any given allostatic pathology to have an impact system wide. The extended amygdala, as an integral part of the extra-hypothalamic stress response systems, is a key structure in allostatic processing based on its functional significance, as well as on the pattern of connectivity inherent in its composition. In fact, the extended amygdala is an integral part of all the neural circuits pertinent to allostasis, and it is well located to drive the allostatic processing in the CNS (Koob and Le Moal, 2001).

4.6 Anatomy of the Extended Amygdala

The extended amygdala is an anatomical theoretical construct based on structural and functional similarities of the participating nuclei (Heimer and Alheid, 1991). The core amygdala nucleus is composed of many intercolated subnuclei that receive input from most brain areas which comes in through the basal and lateral nuclei, and send output to many different brain areas, mostly through central and medial nuclei. The central nucleus of the amygdala (CeA) also sends downstream projections to the brainstem where it can elicit a sympathetic nervous system response. The extended amygdala includes Shell of Nucleus Accumbens (NAcc), Bed Nucleus of Stria Terminalis (BNST or BST), and the Central and Medial Nuclei of the Amygdala based

on cytoarchitectonic and functional similarities of these regions. (Heimer et al., 1997) Other amygdala subnuclei are closely related to this structure based on connectivity patterns and functional similarity. In the framework of extended amygdala, BNST is considered to be an extension of the central nucleus of the amygdala (CeA), while the shell of NAcc is an extension of the medial nucleus of the amygdala (MeA) due to dense bidirectional projections connecting these structures. There is also enough cytoarchitectural similarity between CeA and MeA to consider them as very closely related structurally, if not functionally (Heimer et al., 1997).

The central and medial amygdalar nuclei (CeA and MeA) send projections to the hypothalamus, and the central nucleus sends projections to the autonomic and somatosensory areas of the brainstem. The conventional borders of CeA and MeA extend neurohistochemically into the sublenticular areas (directly below the globus pallidus), such as acetylcholine (Ach) rich substantia inominata (SI) in the basal forebrain, and to the dopamine (DA) rich BNST areas (Heimer et al., 1997). It is through these connections that the CeA has access to the entire cortical mantle. The interconnections between CeA, MeA, BNST, and the sublenticular areas are so prominent that the sublenticular and BNST regions are now referred to as "extended" or "sublenticular" amygdala (Heimer et al., 1997). The connectivity pattern of the sublenticular amygdala is similar to that of the CeA and MeA. Through the direct brainstem and sublenticulo-cortical connections, the CeA is able to modulate neurochemical systems in much of the brain.

4.7 Functional Dysregulation During Allostatic State Conditions

Under conditions of stress and arousal, certain memory processes are altered. The basolateral amygdala (BLA) is important for the enhanced consolidation of affective or stressful memories, while the prefrontal cortex (PFC) is involved in mediating the deleterious effects of stress on working memory. (Birnbaum et al., 1999; Quirarte et al., 1997; Roozendaal, 1999; McGaugh et al., 1992). These neural systems become loci of allostatic processing when their functions and structures are altered in the presence of high neural or environmental demand. The interaction of stressful stimuli from the environment in concert with the increased neural processing in these systems elicit adaptive, or coping responses on both structural and functional levels (McEwen and Seeman, 1999).

4.8 Hippocampus and Allostatic Processing

Hippocampus has been broadly implicated in many facets of cognition including memory, orientation in space, and orientation in time (White and McDonald, 1993; White et al., 1989; Kesner et al., 2004). The spatial memory link has been particularly well researched, with the hippocampus emerging as one of the vital structures for acquiring new spatial information about an environment (White et al., 1989; Kesner et al., 2004; Squire et al., 2004; McDonald and White, 1995).

Hippocampus is also one of key structures that illustrates the effect of dysregulated allostasis on cognitive processing at both functional and structural levels. The hippocampus is directly involved in the CNS stress response, since it receives glucocorticoid input from the periphery (Koob and Le Moal, 2001). Additionally, hippocampus is one of the sites for CRF synthesis (Smith et al., 1997). Production of

CRF is one piece of evidence of the hippocampal involvement in stress. Additionally, CRF mRNA levels in the dentate gyrus of the hippocampus rise noticeably after amygdala kindling (Smith et al., 1997), indicating that the stress response in the hippocampus may be dependent on amygdalar activity. This hypothesis fits well with the conjecture that the extended amygdala drives allostatic processes.

The hippocampus has been one of the more researched sites for neuronal plasticity in the adult brain, due, in part, to several structural changes that have been observed in the hippocampal neurons. These changes include dendritic remodeling in CA3 pyramidal neurons, cell death in the dentate gyrus/granule cell layer, and the genesis of new cells in the granule cell layer which later migrate to the dentate gyrus (Gould et al., 1997; Magariños and McEwen, 1995a; Magariños and McEwen, 1995b; Uno et al., 1989). Dendritic remodeling in the hippocampal pyramidal cells can take two forms, either atrophy of dendrites or more branching and growth of dendritic trees (Magariños and McEwen, 1995a; Magariños and McEwen, 1995b). All of this neuronal plasticity has been associated with changes in spatial memory and is affected by stress, both in the case of experimenter induced as well as naturally occuring social stress (Gould et al., 1997; McEwen, 2000b).

The extent of cell death and neurogenesis in the dentate gyrus has been hypothesized to be regulated by the environmental demands for spatial memory (McEwen, 2000b). High levels of cell turnover in the hippocampus would indicate that the demand for spatial memory usage is increased, and vise versa. However, stressful stimuli can interfere with this relationship (McEwen, 2000b). Dendritic plasticity is

correlated with the richness of habitat and the amount of exploration and can also be modulated by stress (Uno et al., 1989; McEwen, 2000b).

Studies have been conducted on both monkeys and rats to elucidate the impact of chronic stress on the hippocampus. Most of the findings indicate that during chronic stress there are several physiological changes that take place in the dentate gyrus of the hippocampus. There is less neurogenesis, there is dendritic atrophy, and there are fewer pyramidal cells apparent in the dentate gyrus (Gould et al., 1997; Magariños and McEwen, 1995a; Magariños and McEwen, 1995b; Uno et al., 1989). In one example, a social stress situation was enacted where vervet monkeys were placed in a living environment with dominant monkeys (Uno et al., 1989). Following death by natural causes their dentate gyri were examined. A reduced number of pyramidal cells was observed, and associated with their lifetime living environment (Uno et al., 1989). A similar type of cell loss was observed in the "virtual burrow system" (VBS) environment for rats in which a natural social hierarchy is established among the animals (Blanchard et al., 1995). This type of hippocampal atrophy could be explained either by lower than normal levels of neurogenesis, or by increased levels of apoptosis (Gould et al., 1997; Uno et al., 1989). Dendritic atrophy of the dentate gyrus neurons has been documented after rats were subjected to several different chronic stress paradigms, including restraint stress daily for 21 days, and a multiple stress paradigm where rats were subjected to 1 hour restraint, 1 hour shaking, and 30 minute swimming in body-temperature water (Magariños and McEwen, 1995a; Magariños and McEwen, 1995b). Similarly, subordinate rats in the VBS also show dendritic atrophy in the dentate gyrus (Blanchard et al., 1995). While these experiments demonstrate the deleterious effects of stressful

conditions using animal models, these findings should also be considered in light of the chronically depressed patients as well as the patients with PTSD, who demonstrate reduction in the hippocampal volume as measured by MRI scans (Friedman, 1997).

Aspects of cognition that are hippocampus dependent, such as spatial learning and memory, are also detrimentally influenced by chronic stress (Luine et al., 1996). Deficits in spatial memory were demonstrated by measuring spatial memory on a radial 8 arm maze in combination with the same chronic stress paradigm that yielded dendritic atrophy in the DG of rats when used alone. Chronically stressed rats made mistakes in this spatial task significantly earlier than controls (Luine et al., 1996). Furthermore, in longitudinal studies, human subjects show spatial memory deficits that are correlated with increases in HPA activity that progress over 4 to 5 years (McEwen, 2000b). These cognitive deficits could point to the direct involvement of the hippocampus in the allostatic load precipitated by chronic stress.

Both the deficits in spatial memory and the dendritic atrophy found in rats subjected to chronic stress are largely reversible after removal of all stressors. This structural and functional recovery could be indicative of the neuroprotective function of the allostatic processing whereby the organism adjusts to environmental demands (McEwen and Seeman, 1999). Such an adaptive coping response to the stressful environment once again underscores the neural and cognitive plasticity inherent in the CNS.

5. Drug Addiction and Allostatic Processing

Coping, or adaptation, is central to the concept of allostasis. Adaptation to stress can occur not only on structural and behavioral levels, but also on a neurochemical level. Adaptation as a neural process can occur in response to any perturbing stimulus that affects the brain. Drugs of abuse can serve as such a stimulus since they act on the brain directly (Koob and Le Moal, 1997). As opposed to the allostatic processing triggered by stressful stimuli, the allostatic processes triggered by drug use are activated directly through the neurochemical sites of action of the drugs in the reward and reinforcement pathways in the brain (Koob, 1992). This unnatural activation of reward mechanisms triggers adaptive responses in the brain, initially on the cellular and molecular levels, and subsequently on structural and behavioral levels. Too much of a rewarding stimulus, exactly like too much of a stressful stimulus, eventually gives rise to an arousal pathology (Koob and Le Moal, 2001).

In order to view drug addiction as an allostatic process, it is important to examine the mode of drug action in the brain, as well as the behavior involved in drug seeking and administration. Drug intake is a self-reinforcing behavior that, at least initially, triggers feelings of pleasure, or reward (Koob, 1992). There are two aspects of drug abuse that are particularly important to consider with respect to the neural circuitry involved in allostatic processing. These two aspects are drug seeking behavior, or the sequence of actions that leads to drug intake, and the motivational salience of the drug stimuli (Koob and Le Moal, 2001). These are very interrelated and both involve the reward and reinforcement circuitry in the brain. During drug addiction, both of these processes are progressively dysregulated (Roberts et al., 2000). Drug seeking behavior can be characterized as a ritualistic, repetetive sequence of actions. Striatal-Pallidal-thalamic

structures play a significant role in stringing together motor movements and the iteration of action sequences, or habit learning (Graybiel et al., 1994). Such disorders as OCD and Schizophrenia point to the role of striatal-cortical-thalamic loops in tendency, or compulsion, to perform certain behaviors over and over (Graybiel, 1997). Thus, these neural circuits can be implicated in the aspect of drug intake that leads to habitual drug-seeking behavior. The structures involved in the assessment of motivational salience and reward evaluation, as opposed to the rote reinforcing influence of the drugs, are the shell of the NAcc and other constituent parts of the extended amygdala (Koob and Le Moal, 1997; Koob and Le Moal, 2001). Through direct as well as indirect mechanisms drugs influence both the rewarding and the reinforcing neural pathways in the brain (Koob, 1992).

5.1 Psychostimulants and Reward

An important neurochemical link in these functions is the involvement of dopamine (DA). A vast body of literature states that the levels of DA in Nucleus Accumbens are indicative of evaluating reward (White, 1989; Schultz, 1998; Koob, 1992). Electrophysiological recordings of dopaminergic neurons in the nucleus accumbens demonstrate that these neurons fire in response to rewards as well as to the stimuli that predict rewards (Schultz, 1998). The degree of dopaminergic neuron activation is proportional to the reward value of a stimulus (Schultz, 2000). DA receptors in parts of the basal ganglia have been implicated in potentiating the execution of automatic movements (Canales and Graybiel, 2000). Lesioning of dopaminergic input to the striatum results in difficulty in initiation of movement as well as being able to shift

from one type of action to the other (Graybiel, 1997). All psychostimulant drugs act on the dopaminergic system, serving in one way or another as DA agonists (Koob, 1992). Administration of psychostimulant drugs initially causes an overstimulation of the dopaminergic pathways (Koob, 1992; Koob and Le Moal, 1997). This is perceived by the subject as a highly rewarding event. Drugs of abuse other than the psychostimulants involve opiate, serotonin, glutamatergic, and gamma-aminobutyric acid (GABA) systems in addition to DA.

5.2 Reward Disregulation, Dopamine, and Allostasis

Pharmacological interactions between the drugs and their target sites in the brain initiate the processes of adaptation on the molecular level to compensate for the unnatural neurochemical intrusion of the drugs. When the reward and reinforcement circuits receive an overflow of DA agonists consistently over a period of time, DA synthesis is downregulated through negative feedback (Koob, 1992). Following the downregulation of DA synthesis and release, any DA agonist is going to elicit a smaller dopaminergic response compared to dopaminergic activation during a baseline level of DA (Koob, 1992; Koob et al., 1997). This is perceived by the subject as a craving for more drugs. This process of counteradaptation is consistent with a homeostatic regulatory mechanism (Koob et al., 1997). However, if drug administration continues, eventually the DA level is going to be low enough where dopaminergic receptors will begin undergoing a process of sensitization, by initiating abnormally overactive response to any DA agonist (Koob et al., 1997). If these processes are reinterpreted in the framework of allostasis, it could be hypothesized that counteradaptation and sensitization dysregulate the reward mechanism.

One way to describe reward dysregulation is as a change in the degree of motivation to seek drug related stimuli. The shift in motivation stems from the DA downregulation in NAcc (Koob and Le Moal, 1997). Since there is a need for more DA, motivation for obtaining the drugs is higher, while the reward value of a given amount of a drug is decreased. Reward dysregulation also involves increased psychomotor compulsion to take the drug (Koob and Le Moal, 2001; Koob et al., 1997; Roberts et al., 2000). DA receptor sensitization in the striatal-pallidal-thalamic loop leads to increased behavioral response to any stimulus that is temporally connected to drug intake. Influx of DA agonists leads to the downregulation of DA synthesis, which in turn leads to further sensitization of the dopaminergic receptors. This process of reward dysregulation is allostatic in nature since there is a persistent deviation from normal neural function that occurs through adaptation to the environment (Koob and Le Moal, 2001). It has been hypothesized that drug addiction is a form of an allostatic load that stems from prolonged reward dysregulation (Roberts et al., 2000).

5.3 Behavioral Measures of Reward Disregulation

Reward dysregulation can be detected by measuring changes in the threshold of reward in drug addicted animals (Markou and Koob, 1991). Behaviorally, this involves measuring the levels of intracranial self stimulation (ICSS). Animals will usually press a lever at a baseline rate to deliver a small amount of electrical current when the stimulating electrode is implanted in the medial forebrain bundle (MFB). The MFB contains DA pathways from the ventral tegmental area to the NAcc, so pressing the lever stimulates a reward pathway in the brain. The lever pressing behavior is repetitive, so it

can be determined if drug addicted animals also have perseverative psychomotor tendencies (Koob, 1992). ICSS can be used in combination with drug administration or NT agonist or antagonist administration. ICSS results can be influenced through modification of certain behavioral parameters, such as testing lever pressing responses in dose dependent versus rate dependent drug administration paradigms. The ICSS experiments can be used to assess psychomotor activity, such as that seen during drug seeking behaviors, as well as the evaluation of reward, or the motivational salience of DA pathway stimulation. The baseline rate of ICSS for drug addicted animals is much higher than that of controls (Markou and Koob, 1991; Koob, 1992). This indicates that these animals need more stimulation to perceive the same level of reward as controls, implying an increase in motivation, or "reward threshold". There is no longer an active homeostatic set-point for perceiving reward, instead the threshold for registering that stimuli are rewarding is continually raised through allostatic dysregulation.

5.4 Stress and Reward – Neurochemical Connection

Another aspect of drug addiction that ties it more closely to the stress induced allostatic load, is the neurochemical involvement of the brain stress systems. Before drug abuse progresses to the level of drug addiction, the levels of CRF rise gradually, and then remain chronically upregulated if the drug addiction persists (Koob and Le Moal, 2001; Roberts et al., 2000). The stress response is hypothesized to become activated through the negative affective state of withdrawal and "wanting" to attain more drug. The behavioral and emotional symptoms of withdrawal may stem from persistent dopaminergic dysfunction (Koob and Le Moal, 1997). Experimentally, CRF injected into

the ventricles will increase the reward threshold for self-stimulation, similar to the change seen in drug exposed and drug addicted animals (Macey et al., 2000). The interaction between the dopaminergic and the CRF systems is crucial for this behavioral state to occur. The extended amygdala is the most likely neural structure where these neuromodulators interact causally.

6. Noradrenergic System and Allostatic Processing Dysregulation of reward is for the most part tied to the dysregulation of the dopaminergic system. Another monoamine, norepinephrine, is also implicated in allostasis through it's influence on stress and arousal mechanisms, since NE is synthesized and secreted in response to stressful and arousing stimuli (Marrocco et al., 1994; Abercrombie and Zigmond, 1995). NE acts in the brain to alert the organism to the presence of stimuli in the environment (Foote et al., 1991). This is accomplished, in part, through NE influencing orienting behavior and attentional processes. NE is produced in several sites in the brainstem, mostly in the Locus Coeruleus (LC), but also in several other cell clusters at the level of the pons, including Nucleus of the Solitary Tract (NTS). LC sends massive projections that innervate most of the functional systems of the brain including both subcortical and cortical structures (Valentino and Aston-Jones, 1995). Importantly, these connections include major inputs to BNST, Amygdala, and the prefrontal cortex (PFC) (Koob, 1999). In turn, NE neurons receive inputs from many of the CRF producing sites, including PVN, BNST, and CeA (Van Bockstaele et al., 1998). It has been demonstrated that in several if not all of these areas, CRF projections potentiate the effects of norepinephrine under conditions of stress (Smagin et al., 1997; Sands et al., 2000). Some of the effects of CRF in the amygdala

and the prefrontal cortex are mediated and influenced by action of NE (Quirarte et al., 1997; Kawahara et al., 2000; Jedema et al., 1999; Birnbaum et al., 1999).

The bidirectional projections between the LC and other, CRF producing, structures involved in the stress response are potentiating in nature, thereby increasing the synthesis and secretion of NE and/or CRF during prolonged activation of these structures (Smagin et al., 1997; Koob, 1999). Thus, during a stress response, it is hypothesized that the central NE and CRF circuits stimulate each other, with CRF potentiating NE levels and vice versa. This hypothesis is called the CRF-NE-CRF feed forward system, and it is based on the presence of the positive feedback loops connecting the NE and CRF producing structures described above (Koob, 1992). It is also plausible, given this hypothesis, that under chronic stress the CRF-NE-CRF feed forward system is one of the mechanisms that would globally and persistently upregulate the levels of NE and CRF in the brain, possibly resulting in an allostatic load condition.

6.1 Noradrenergic effect on the amygdala function

The increase in NE synthesis and secretion in the amygdala during stress is CRF dependent, although NE levels can also become elevated in response to general physiological arousal through mechanisms independent of the stress response (Charney et al., 1995; Ferry et al., 1999c; Quirarte et al., 1997; Roozendaal, 2000; Setlow et al., 2000). Multiple studies have been done to elucidate the role of the amygdala in affective arousal and memory. Rats trained on retention memory tasks show enhanced memory if a physiologically arousing drug is injected systemically during the acquisition phase of the experiment (McGaugh et al., 1996; McGaugh et al., 1992; Roozendaal, 2000). Lesions

or transient inactivations of amygdala abolish the memory enhancing effect of drugs and hormones that induce arousal (Cahill et al., 1996; McGaugh et al., 1996; McGaugh et al., 1992; Roozendaal, 2000). This reduction in memory is observed if the amygdala is lesioned prior to the acquisition stage, but not prior to the performance stage. This finding is valid for both general, epinephrine induced, arousal, and the arousal induced by the glucocorticoids during stress. Studies of humans with pre-existing brain damage conditions limited to lesions of the amygdala show the same lack of memory enhancement for emotionally arousing information (Cahill et al., 1995). It has been concluded that amygdala serves as a modulator of emotionally significant events, and is an important neural component in processing these events (McGaugh et al., 1992, Cahill et al., 1996).

Specifically, using an inhibitory avoidance behavioral paradigm, it has been shown that memory for a stressful experience (mild electric shock) can be influenced by both CRF and NE modifications in the BLA (Roozendaal, 2000; Ferry et al., 1999c). Both NE administered directly into the BLA and CRF administered intercerebroventricularly (ICV) enhance memory for a stressful experience. Experiments with localized administration of adrenergic receptor antagonists alone, or in combination with glucocorticoid receptor agonists, immediately after the stressor, have shown that the enhancing effect of stress on emotional memories is mediated through the beta and alpha adrenergic receptor mechanisms in the BLA (Ferry et al., 1999a; Ferry et al., 1999b; Ferry et al., 1999c).

7. Stress and PFC function As compared to memories for arousing and stressful events, the on-line processing of information, or active remembering of events, termed working memory, is adversely affected by stress (Birnbaum et al., 1999). Working memory is partially mediated by the prefrontal cortex, another structure that receives input from both NE and CRF producing structures. PFC is important in working memory tasks that require some part of information to be kept "on line", or actively remembered, in order to be able to solve a subsequent task. PFC is interconnected with many other structures involved in allostatic processing, including the amygdala, striatal-pallidal-thalamic loops, the hippocampus, and the basal forebrain (Heimer and Alheid, 1991). By virtue of these connectivity patterns, PFC is an integral structure in mediating the effects of allostasis on working memory. Since stress is one of the conditions that upregulates allostatic processing, it is important to consider the effects of stress on PFC both neurochemically and functionally.

7.1 Noradrenergic effect on the PFC function

Following acute stress, NE levels in the PFC increase significantly, a phenomenon directly mediated by CRF dependent mechanisms (Kawahara et al., 2000; Jedema et al., 1999). Administration of a direct CRF antagonist prevents the rise in NE that normally occurs following a stressful experience (Smagin et al., 1997). The performance on working memory paradigms subserved by the PFC is negatively affected by stress. This has been demonstrated using various working memory paradigms with primates and rats exposed to mild or moderate stress (Birnbaum et al., 1999). The deletarious effect of stress on working memory is mediated through noradrenergic

mechanisms. This has been shown in a T-maze working memory paradigm in rats, using a systemically administered pharmacological agent as a stressful stimulus. Rats will perform poorly, displaying both less accuracy and more perseveration in this task if they are exposed to this stressor. Administration of a selective NE antagonist directly into the PFC blocks the effect of the stressor, returning the rat's performance to baseline levels (Birnbaum et al., 1999). Thus, naturally high levels of NE that are present in PFC during a stress response and that depend on CRF upregulation, adversely affect working memory function.

A general hypothesis to explain this phenomenon from a perspective of cognitive processing is that during on-line processing of information, cells in the PFC need to be active during the delay between trials, however, if there is extra NE present because of stress, it will induce glucocorticoid release in PFC and increase cell activity, thereby decreasing the normal signal to noise ratio. DA is also involved in this hypothesis - postulating that extra DA in the synapse will interfere with Ca2+ delivery into the cell (Birnbaum et al., 1999). From the viewpoint of allostasis, however, this can be more broadly explained as the effect of allostatic processing on cognitive function. Additionally, the incidence of behavioral perseveration in rats during a working memory task could potentially indicate the recruitment of the basal ganglia function precipitated through initiation of the allostasic processing.

In summary, NE and CRF acting in concert seem to be potentiating an allostatic load condition in several brain systems, with amygdala and PFC memory functions being just the two examples discussed above. In terms of system wide NE upregulation during stress, increased NE in the amygdala enhances affective memory consolidation

(Roozendaal, 2000). On the contrary, increased NE levels, as well as decreased glucocorticoid levels, in the PFC interfere with the working memory tasks normally mediated by the PFC (Birnbaum et al., 1999).

7.2 Glucocorticoid effect on the PFC function

Endogenous glucocorticoid levels are vital for PFC dependent cognitive performance, revealed by both spatial, and non-spatial working memory tasks (Mizoguchi et al., 2004; Cerqueira et al, 2005). Altered levels of glucocorticoids in PFC, either through chronic behavioral stress or caused by adrenalectomies, lead to poor performance on working memory tasks, and are accompanied by structural changes in PFC (Mizoguchi et al, 2000; Mizoguchi et al, 2001; Mizoguchi et al., 2004; Cerqueira et al, 2005). Importantly, bilateral lesions of the BLA have been shown to relieve these stress mediated PFC dependent memory impairments (Roozendaal et al., 2004).

In both the amygdala and the PFC, CRF is highly involved in mediating the stress response. In the case of PFC function during stress, NE upregulation is CRF dependent as shown by the behavioral paradigms discussed above. In the amygdala, increases in CRF potentiate the noradrenergic mechanisms for the consolidation of affective memories. The CRF-NE-CRF feed forward system hypothesis states that during chronic stress both CRF and NE levels rise above the baseline due to the repeated reactivation of the positive feedback loops that connect the CRF and NE producing structures (Koob, 1999). The integration of this hypothesis together with what is known about the neurochemical interactions of NE and CRF in the amygdala and the PFC, suggests that during allostatic state conditions information processing in the PFC is impaired, while the

functions subserved by the amygdala are highly enhanced. Furthermore, the impairment in PFC function is amygdala dependent (Roozendaal et al., 2004).

Additionally, effects of altered endogenous glucocoirticoid levels, achieved through adrenalectomies, on PFC function involve changes in Dopamine receptor production (Mizoguchi et al., 2002; Mizoguchi et al., 2004). While the binding patterns of D1 receptors in PFC remain normal, the density of D1 receptors is drastically increased, as a consequence of decreased DA release from the VTA (Mizoguchi et al., 2004).

8. Discussion and Conclusion

On the level of neural systems, it can be implied that stress has contrasting effects on functions at cortical as opposed to subcortical levels. One of the inferences is that during stress, a kind of "de-evolution" is taking place where the processing of information subserved by the cortical structures, like the PFC, is impaired. On the other hand, functions that include assessment of the environment and behavioral responses to stressful situations are subserved by subcortical structures, like the amygdala and the Basal Ganglia. During stress these structures show enhanced functioning - strengthening memory for stressful events in the process. It has been argued that this approach is in fact an evolutionarily adaptive strategy for surviving in adverse environments (Arnsten, 1998). This statement stems from the notion that in stressful, adverse situations, especially those that elicit SNS response, being able to concentrate and perform working memory tasks is not as crucial as responding behaviorally to avoid the situation and remembering what the situation involved to be able to avoid it in the future.

The strength and extent of connections between the neural structures implicated in allostasis underscores the importance of the CNS control of allostatic processing. The extended amygdala, connected to all of the systems affected by stress and drug addiction, is in an advantageous position to drive allostatic processing. Furthermore, the positive feedback of stress hormones on the extended amygdala is additionally important to its ability to regulate allostasis, since this feedback supplies information about the level of stress and arousal in the brain and body at any given time (Gray, 1993; Koob and Le Moal, 2001). This information also enables anticipatory action by the extended amygdala and associated structures to prepare for further demands on the brain and body. The evidence presented earlier detailed how chronic stress plays an important role in hippocampal function, compromising spatial memory in chronically stressed rats and contributing to structural reorganization in the hippocampus (Luine et al., 1996; Magariños and McEwen, 1995a; Magariños and McEwen, 1995b). This research on social stress and its capability to elicit physiological damage in the brain is also consistent with the allostatic framework (Virgin and Sapolsky, 1997; Uno et al., 1989; Vellucci, 1990). Connections between the hippocampus and the amygdala are relevant in a stress response, since kindling in the amygdala stimulates CRF gene expression in the hippocampus (Smith et al., 1997). All of these interactions between the CNS stress response and other functions subserved by these structures emphasize the global role for allostasis as a powerful biological regulatory mechanism.

Sterling and Eyer, in their original work implied that the conceptual model of allostasis can be broadly applied, to explain the functional stability of virtually all the different parameters in an organism (Sterling and Eyer, 1988). Some of the explicit links

that Sterling and Eyer brought up were the connections between the brain and the immune system, and how psychological factors are inextricably tied to the physiological ones (1988). Shulkin et al. picked up on this hypothesis and applied it specifically to neural processing (1994). Shulkin et al. also emphasized the immense strain that the dysregulation of allostasis would cause on the brain and through it, on the body (1994). Up through this time in the development of the allostasis theory, the most important causal factor for allostatic processing and dysregulation was stress.

Following the proposal of the preceeding ideas, Koob and collegues proposed a more expansive model for neural control of allostasis, centering it on the hypothesis that drug addiction is a different form of allostatic load, that recruits the involvement of the stress systems as an aftereffect (Koob and Le Moal, 2001; Roberts et al., 2000). All of these implications fit easily into the conceptual framework of allostasis, as well as specifically neural control and neurally based instances of allostasis.

Further, the co-morbidity of symptomps in many mental disorders exhibited by humans, as well as the overlap in some of the physiological damage observed, underscore the importance of the connectivity patterns of neural substrates in these disorders. In fact, if a mental illness that results from the dysregulation of a certain neural mechanism results in a condition of allostatic load, there is good reason to argue that other structures and functions involved in allostatic processing become susceptible to the dysregulation as well. This is the global state of vulnerability of neural systems that is conferred by allostatic load. This is also one means of explaining the co-morbidity in many of the mental disorders, as well as the tendency of mental patients, such as schizophrenics or PTSD patients, to become susceptible to substance abuse, for instance nicotine and

alcohol (Friedman, 1997; McEwen, 2000b). Thus, there is a broad implication for the interrelatedness of the mood and anxiety disorders that stems directly from the interconnectedness of the neural circuits involved in allostasis.

Participation of other neurotransmitters and neuromodulators including Glu, GABA, Ach, and 5HT is also important in allostatic processing (Koob and Le Moal, 2001; McEwen, 2000a; McEwen, 2000b). All of these neurochemicals are involved in mediating functions in many of the structures implicated in neural allostatic control. The role of these neurochemicals in allostasis, as well as the broad field of research concerning the impact of allostasis and allostatic load on the immune system and on sleep are currently beyond the scope of this chapter.

9. References

Abercrombie, Elizabeth D.; Zigmond, Michael J.. Modification of central catecholaminergic systems by stress and injury: Functional significance and clinical implications. Bloom, F. E. Kupfer, D. J., In: Psychopharmacology: The fourth generation of progress. Raven Press ; 1185 Avenue of the Americas, New York, New York 10036-2806, USA, 1995. 355-361.

Allsworth JE, Weitzen S, Boardman LA. Early age at menarche and allostatic load: data from the Third National Health and Nutrition Examination Survey. Ann Epidemiol. 2005 Jul;15(6):438-44.

Arnsten, AF. The biology of being frazzled. Science, 1998 Jun 12, 280(5370):1711-2.

Bay E, Kirsch N, Gillespie B. Chronic stress conditions do explain posttraumatic brain injury depression. Res Theory Nurs Pract. 2004 Summer-Fall;18(2-3):213-28.

Birnbaum, S; Gobeske, KT; Auerbach, J; Taylor, JR; Arnsten, AF. A role for norepinephrine in stress-induced cognitive deficits: alpha-1-adrenoceptor mediation in the prefrontal cortex. Biological Psychiatry, 1999 Nov 1, 46(9):1266-74.

Blanchard, DC; Spencer, RL; Weiss, SM; Blanchard, RJ; McEwen, B; Sakai, RR. Visible burrow system as a model of chronic social stress: behavioral and neuroendocrine correlates. Psychoneuroendocrinology, 1995, 20(2):117-34.

Blanchard, RJ; Nikulina, JN; Sakai, RR; McKittrick, C; McEwen, B; Blanchard, DC. Behavioral and endocrine change following chronic predatory stress. Physiology and Behavior, 1998 Feb 15, 63(4):561-9.

Burchfield, SR. The stress response: a new perspective. Psychosomatic Medicine, 1979 Dec, 41(8):661-72.

Cahill, L., Haier, R.J., Fallon, J., Alkire, M.T., Tang, C., Keator, D., Wu, J., McGaugh, J.L. Amygdala activity at encoding correlated with long-term, free recall of emotional information. Proceedings of the National Academy of Sciences,USA, 1996, 93, 8016-8021.

Cahill,L.F., Babinsky ,R., Markowitsch,H.J., McGaugh, J.L.: The amygdaloid complex and emotional memory. Nature 1995, Sep 28, 377(6547):295-6.

Canales, JJ; Graybiel, AM. A measure of striatal function predicts motor stereotypy. Nature Neuroscience, 2000 Apr, 3(4):377-83.

Charney, Dennis S.; Bremner, J. Douglas; Redmond, D. Eugene,. Noradrenergic neural substrates for anxiety and fear: Clinical associations based on preclinical research. Bloom, F. E. Kupfer, D. J., In: Psychopharmacology: The fourth generation of progress. Raven Press ; 1185 Avenue of the Americas, New York, New York 10036-2806, USA, 1995. 387-395.

Cole, BJ; Koob, GF. Propranolol antagonizes the enhanced conditioned fear produced by corticotropin releasing factor. Journal of Pharmacology and Experimental Therapeutics, 1988 Dec, 247(3):902-10.

Curtis AL, Bello NT, Connolly KR, Valentino RJ. Corticotropin-releasing factor neurones of the central nucleus of the amygdala mediate locus coeruleus activation by cardiovascular stress. J Neuroendocrinol. 2002 Aug;14(8):667-82.

Davis, M. The role of the amygdala in emotional learning. International Review of Neurobiology, 1994, 36:225-66.

Ferry B, Roozendaal B, McGaugh JL (1999a): Basolateral amygdala noradrenergic influences on memory storage are mediated by an interaction between β- and α1-adrenoceptors. J Neurosci 19:5119-5123..

Ferry B, Roozendaal B, McGaugh JL (1999b): Involvement of the α1-adrenergic receptors in the basolateral amygdala in modulation of memory storage. Eur J Pharmacol 372:9-16..

Ferry B, Roozendaal B, McGaugh JL. Role of norepinephrine in mediating stress hormone regulation of long-term memory storage: a critical involvement of the amygdala. Biological Psychiatry. 1999c Nov 1;46(9):1140-52.

Foote, SL; Berridge, CW; Adams, LM; Pineda, JA. Electrophysiological evidence for the involvement of the locus coeruleus in alerting, orienting, and attending. Progress in Brain Research, 1991, 88:521-32.

Friedman, MJ. Posttraumatic stress disorder. Journal of Clinical Psychiatry, 1997, 58 Suppl 9:33-6.

Galambos SA, Terry PC, Moyle GM, Locke SA, Lane AM. Psychological predictors of injury among elite athletes. Br J Sports Med. 2005 Jun;39(6):351-4; discussion 351-4.

Galvez, R., Mesches, M. and McGaugh, J.L., 1996. Norepinephrine release in the amygdala in response to footshock stimulation. Neurobiol Learn Mem 66, pp. 253-257.

Gould, E; B.S. McEwen, P. Tanapat, L.A.M. Galea and E. Fuchs, Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. J. Neurosci. 17 (1997), pp. 2492-2498.

Gray, TS. Amygdaloid CRF pathways. Role in autonomic, neuroendocrine, and behavioral responses to stress. Annals of the New York Academy of Sciences, 1993 Oct 29, 697:53-60.

Graybiel, AM. The basal ganglia and cognitive pattern generators. Schizophrenia Bulletin, 1997, 23(3):459-69.

Graybiel, AM; Aosaki, T; Flaherty, AW; Kimura, M. The basal ganglia and adaptive motor control. Science, 1994 Sep 23, 265(5180):1826-31.

Heimer L, Alheid GF. Piecing together the puzzle of basal forebrain anatomy. Advanced Experimental Medical Biology. 1991;295:1-42.

Heimer, L; Alheid, GF; de Olmos, JS; Groenewegen, HJ; Haber, SN; Harlan, RE; Zahm, DS. The accumbens: beyond the core-shell dichotomy. Journal of Neuropsychiatry and Clinical Neurosciences, 1997 Summer, 9(3):354-81.

Hugdahl, K. Cognitive influences on human autonomic nervous system function. Current Opinion in Neurobiology, 1996 Apr, 6(2):252-8.

Jedema, HP; Sved, AF; Zigmond, MJ; Finlay, JM. Sensitization of norepinephrine release in medial prefrontal cortex: effect of different chronic stress protocols. Brain Research, 1999 Jun 5, 830(2):211-7.

Kawahara, H; Kawahara, Y; Westerink, BH. The role of afferents to the locus coeruleus in the handling stress-induced increase in the release of noradrenaline in the medial prefrontal cortex: a dual-probe microdialysis study in the rat brain. European Journal of Pharmacology, 2000 Jan 17, 387(3):279-86.

Kesner RP, Lee I, Gilbert P. A behavioral assessment of hippocampal function based on a subregional analysis. Rev Neurosci. 2004;15(5):333-51.

Koob GF. Neuroadaptive mechanisms of addiction: studies on the extended amygdala. Eur Neuropsychopharmacol. 2003 Dec;13(6):442-52.

Koob, G.F. and Le Moal, M., 1997. Drug abuse: Hedonic homeostatic dysregulation. Science 278, pp. 52-58.

Koob, GF. Corticotropin-releasing factor, norepinephrine, and stress. Biological Psychiatry, 1999 Nov 1, 46(9):1167-80.

Koob, GF. Neural mechanisms of drug reinforcement. Annals of the New York Academy of Sciences, 1992 Jun 28, 654:171-91.

Koob, GF; Caine, SB; Parsons, L; Markou, A; Weiss, F. Opponent process model and psychostimulant addiction. Pharmacology, Biochemistry and Behavior, 1997 Jul, 57(3):513-21.

Koob, GF; Le Moal, M. Drug Addiction, Dysregulation of Reward, and Allostasis. Neuropsychopharmacology, 2001 Feb, 24(2):97-129.

Luine, V; Martinez, C; Villegas, M; Magariños, AM; McEwen, BS. Restraint stress reversibly enhances spatial memory performance. Physiology and Behavior, 1996 Jan, 59(1):27-32.

Macey, DJ; Koob, GF; Markou, A. CRF and urocortin decreased brain stimulation reward in the rat: reversal by a CRF receptor antagonist. Brain Research, 2000 Jun 2, 866(1-2):82-91.

Magariños, AM; McEwen, BS. Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: comparison of stressors. Neuroscience, 1995a Nov, 69(1):83-8.

Magariños, AM; McEwen, BS. Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: involvement of glucocorticoid secretion and excitatory amino acid receptors. Neuroscience, 1995b Nov, 69(1):89-98.

Markou, A; Koob, GF. Postcocaine anhedonia. An animal model of cocaine withdrawal. Neuropsychopharmacology, 1991 Jan, 4(1):17-26.

Marrocco, RT; Witte, EA; Davidson, MC. Arousal systems. Current Opinion in Neurobiology, 1994 Apr, 4(2):166-70.

McDonald, R. J., & White, N. M. (1995). Information acquired by the hippocampus interferes with acquisition of the amygdala-based conditioned-cue preference in the rat. Hippocampus, 5(3), 189-197.

McEwen, B.S. The neurobiology of stress: from serendipity to clinical relevance. Brain Research, 886(1-2), Dec. 2000(b), pp.172-189.

McEwen, Bruce S.; Seeman, Teresa Protective and damaging effects of mediators of stress: Elaborating and testing the concepts of allostasis and allostatic load. In: Nancy E. Adler, Ed; Michael Marmot, Ed; et al. Socioeconomic status and health in industrial nations: Social, psychological, and biological pathways.. New York Academy of Sciences: New York, NY, US, 1999. p. 30-47 of xv, 503pp.

McEwen, BS. Allostasis and allostatic load: implications for neuropsychopharmacology [see comments] Neuropsychopharmacology, Feb 2000(a), 22(2):108-24.

McEwen, BS. Stress, adaptation, and disease. Allostasis and allostatic load. Annals of the New York Academy of Sciences, 1998 May 1, 840:33-44.

McEwen, BS; Biron, CA; Brunson, KW; Bulloch, K; Chambers, WH; Dhabhar, FS; Goldfarb, RH; Kitson, RP; Miller, AH; Spencer, RL; Weiss, JM. The role of adrenocorticoids as modulators of immune function in health and disease: neural, endocrine and immune interactions. Brain Research. Brain Research Reviews, 1997 Feb, 23(1-2):79-133.

McEwen, BS; Sapolsky, RM. Stress and cognitive function. Current Opinion in Neurobiology, 1995 Apr, 5(2):205-16.

McGaugh, J.L., Introini-Collison, I.B., Cahill, L., Kim, M., Liang, K.C.: Involvement of the amygdala in neuromodulatory influences on memory storage. In The Amygdala. Edited by JP Aggleton. New York City: Wiley-Liss; 1992:431-452.

McGaugh, JL, Cahill, L, Roozendaal, B: Involvement of the amygdala in memory storage: Interaction with other brain systems. Proc. Natl. Acad. Sci. USA, 1996, 93:13508-13514

Michelson, David; Gold, Philip W. Pathophysiologic and somatic investigations of hypothalamic-pituitary-adrenal axis activation in patients with depression. In: Samuel M. McCann, Ed; James M. Lipton, Ed; et al. Annals of the New York Academy of Sciences, Vol. 840: Neuroimmunomodulation: Molecular aspects, integrative systems, and clinical advances.. New York Academy of Sciences: New York, NY, USA, 1998. p. 717-722 of xiv, 866pp. Series title: Annals of the New York Academy of Sciences, Vol. 840.

Quirarte, G.L., Roozendaal, B. and McGaugh, J.L., 1997. Glucocorticoid enhancement of memory storage involves noradrenergic activation in the basolateral amygdala. Proceedings of the National Academy of Sciences, USA. 1997 Dec 9, 94(25):14048-53.

Roberts, AJ; Heyser, CJ; Cole, M; Griffin, P; Koob, GF. Excessive ethanol drinking following a history of dependence: animal model of allostasis. Neuropsychopharmacology, 2000 Jun, 22(6):581-94.

Roozendaal, B. 1999 Curt P. Richter award. Glucocorticoids and the regulation of memory consolidation. Psychoneuroendocrinology, 2000 Apr, 25(3):213-38.

Sands, SA; Strong, R; Corbitt, J; Morilak, DA. Effects of acute restraint stress on tyrosine hydroxylase mRNA expression in locus coeruleus of Wistar and Wistar-Kyoto rats. Brain Research. Molecular Brain Research, 2000 Jan 10, 75(1):1-7.

Sapolsky, Robert M. Potential behavioral modification of glucocorticoid damage to the hippocampus. Behavioural Brain Research. 1993 Nov. 57 (2): p. 175-182

Sapolsky, Robert M.; Share, Lisa J. Rank-related differences in cardiovascular function among wild baboons: Role of sensitivity to glucocorticoids. American Journal of Primatology. 1994. 32 (4): p. 261-275

Schulkin, J; Gold, PW; McEwen, BS. Induction of corticotropin-releasing hormone gene expression by glucocorticoids: implication for understanding the states of fear and anxiety and allostatic load. Psychoneuroendocrinology, 1998 Vol. 23, No. 3, pp219-243.

Schulkin, J; McEwen, BS; Gold, PW. Allostasis, amygdala, and anticipatory angst. Neuroscience and Biobehavioral Reviews, 1994 Fall, 18(3):385-96

Schultz, W. Predictive reward signal of dopamine neurons. Journal of Neurophysiology, 1998 Jul, 80(1):1-27.

Schultz, W. Multiple reward signals in the brain. Nat Rev Neurosci. 2000 Dec;1(3):199-207.

Seeman TE, McEwen BS, Rowe JW, Singer BH. Allostatic load as a marker of cumulative biological risk: MacArthur studies of successful aging. Proc Natl Acad Sci U S A. 2001 Apr 10;98(8):4770-5. Epub 2001 Apr 3.

Selye, H. A syndrome produced by diverse noxious agents. Nature 1936; 32:138

Setlow, B; Roozendaal, B; McGaugh, JL. Involvement of a basolateral amygdala complex-nucleus accumbens pathway in glucocorticoid-induced modulation of memory consolidation. European Journal of Neuroscience, 2000 Jan, 12(1):367-75.

Smagin, GN; Zhou, J; Harris, RB; Ryan, DH. CRF receptor antagonist attenuates immobilization stress-induced norepinephrine release in the prefrontal cortex in rats. Brain Research Bulletin, 1997, 42(6):431-4.

Smith, MA; Weiss, SR; Berry, RL; Zhang, LX; Clark, M; Massenburg, G; Post, RM. Amygdala-kindled seizures increase the expression of corticotropin-releasing factor (CRF) and CRF-binding protein in GABAergic interneurons of the dentate hilus. Brain Research, 1997 Jan 16, 745(1-2):248-56.

Squire LR, Stark CE, Clark RE. The medial temporal lobe. Annu Rev Neurosci. 2004;27:279-306.

Sterling, Peter; Eyer, Joseph Allostasis: A new paradigm to explain arousal pathology. In: Shirley Fisher, Ed; James Reason, Ed; et al. Handbook of life stress, cognition and health. John Wiley & Sons: Chichester, England UK, 1988. p. 629-649 of xxxiii, 750pp.

Uno, H; Tarara, R; Else, JG; Suleman, MA; Sapolsky, RM. Hippocampal damage associated with prolonged and fatal stress in primates. Journal of Neuroscience, 1989 May, 9(5):1705-11.

Valentino, Rita J.; Aston-Jones, Gary S.. Physiological and anatomical determinants of locus coeruleus discharge: Behavioral and clinical implications. Bloom, F. E. Kupfer, D. J., In: Psychopharmacology: The fourth generation of progress. Raven Press ; 1185 Avenue of the Americas, New York, New York 10036-2806, USA, 1995. 373-385.

Van Bockstaele, E.J., Colago, E.E. and Valentino, R.J., 1998. Amygdaloid corticotropin-releasing factor targets locus coeruleus dendrites: Substrate for the coordination of emotional and cognitive limbs of the stress response. J Neuroendocrinol 10, pp. 743-757.

Vellucci, SV. Primate social behavior--anxiety or depression? Pharmacology and Therapeutics, 1990, 47(2):167-80.

Virgin, Charles E. Jr.:; Sapolsky, Robert M. Styles of male social behavior and their endocrine correlates among low-ranking baboons. American Journal of Primatology. 1997. 42 (1): p. 25-39.

White NM, McDonald RJ. Acquisition of a spatial conditioned place preference is impaired by amygdala lesions and improved by fornix lesions. Behav Brain Res. 1993 Jun 30;55(2):269-81

White, N. M. (1989). Reward or reinforcement: what's the difference? Neurosci Biobehav Rev, 13(2-3), 181-186

V

Behavioral Context and Physiological Arousal

1. Abstract

Amygdalar subnuclei, such as the central and the basolateral components of the amygdala, are extensively interconnected not only with the brain circuitry subserving learning and motivation, but also with structures in the brain that regulate endocrine and cardiovascular processes. The existence of communication between the structures that enable behavioral, cognitive, hormonal and autonomic functionality strongly suggests the importance of interaction between the functions themselves, as well as the importance of studying and assessing these processes simultaneously. This chapter details the assessment of two of these functions – the level of cardiovascular response and its interaction with contingent behaviors and learning. The studies conducted effectively argue that cardiovascular activity, represented by mean blood pressure, predictably changes during a contextual switch from a non-contingent to a contingent behavioral environment. This was accomplished by comparing a baseline non-contingent conditioned behavior to a learning behavior in an amygdala dependent conditioned place preference paradigm. In order to elucidate the plausibility and the extent of interaction between behavior and autonomic response, an anatomical model of interconnectivity between the learning, motivational, and autonomic neural circuitries.

2. Introduction

Affect and preference are powerful motivators of associative learning. The amygdala, and, specifically, the basolateral complex of the amygdala (BLA), plays a

crucial role in affective associative learning (Everitt et al., 1989; Everitt et al., 1991; Hiroi & White, 1989; Davis, 1992: Gallagher and Chiba, 1996; Hatfield et al., 1996; LeDoux, 1986; Balleine et al., 1997; Killcross et al., 1997; Malkova et al., 1997; Whalen, 1998). Associative learning is usually expressed through overt behavior; however, affectively motivated learning is often reflected physiologically as well as behaviorally. For example, there are fairly well established changes in cardiovascular parameters during various fear conditioning paradigms (Iwata and LeDoux, 1988; Carrive, 2000). Moreover, increased physiological arousal is generally accepted as indicative of the affective state of the organism. Measuring physiological arousal during associative learning can therefore potentially inform us of the affective state of the organism, as well as give an indication of the extent of involvement of physiological arousal in learning.

The cardiovascular response is one of the most common measurable correlates of the extent of physiological arousal. It has been proposed that affect has at least two key elements: valence, which is a positive or aversive sensory component, and arousal, a high or low level of physiological response (Lang, 1995, Gallagher and Chiba, 1996). Heart rate, as one measure of physiological arousal, has provided an index in a variety of behavioral conditioning paradigms (Hunt and Campbell, 1997; Kostarczyk and Fonberg, 1982; Frysztak and Neafsey, 1994; LeDoux, 1993; Taylor et al., 1994; Abdeen et al., 1995). Heart rate changes have also been associated with both basolateral and central amygdala function (Gallagher et al., 1982; Young and Leaton, 1996; Powell et al., 1997; Kapp et al., 1979; Soltis et al., 1997; Shekhar et al., 1999; Sajdyk and Shekhar, 1997; Roozendaal et al., 1999b; Miyashita and Williams, 2002).

As sited above, there exists a body of research that explores how the brain directs and controls expression of associative learning. There is also much research dedicated to understanding how neural processes regulate physiological responses, including those responses additionally mediated by conditioning, such as heart rate and blood pressure. Still, the question of how neural circuitry that regulates associative learning interacts with the neural circuitry that participates in regulation of physiological measures remains largely open. In order to lay the groundwork for answering this question, we embarked on an experimental design for testing how the neural structures, such as BLA, involved separately in both associative learning and physiological control could potentially play a significant role in regulation of both learning and physiology, effectively linking the separate neuroanatomical circuits. Conversely, the potential interaction of learning and physiological response could bear influence not only on the subserving neural circuitry, but also on resultant behavior, the extent of which can be tested experimentally.

In this experiment, the degree of BLA involvement in baseline modulation of cardiovascular response was assessed during non-contingent appetitive behavior in rats. To accomplish this, heart rate and blood pressure were directly recorded in awake behaving rats freely eating freely available cereal in a contained (pot) environment. In addition, the BLA was temporarily inactivated through the transient NMDA channel blocking effect of the AP5 infusions or with (vehicle) aCSF infusions, A bedding filled plastic flower pot served as the contained “baseline” environment.

In addition, the cardiovascular response during a context switch from the baseline environment into the “learning environment” was measured. The task in this environment was a form of Conditioned Place Preference (CPP, a BLA dependent

learning task) concurrent with either BLA inactivation or a functional BLA. Heart rate (HR) and blood pressure (BP) were recorded while rats explored the baseline environment and searched out and consumed sweet cereal. In the continuation of the same trial, HR and BP were recorded while the animals were trained on a Conditioned Place Preference task (a BLA dependent learning task) in a three-arm maze, and then transferred back to the baseline environment.

Finally, we measured the cardiovascular response during a context switch from the baseline environment into the "learning environment" where rats were tested on retention of CPP (a BLA dependent learning task). HR and BP were measured while rats explored the baseline environment and searched out and consumed sweet cereal. Subsequently, the animals were transferred to the three arm maze and tested on retention of CPP, acquired 48 hours previously. Lastly, the rats were transferred back to the baseline environment Kesner et al., 1989 with HR and BP recorded throughout.

Finally, a neuroanatomical framework is defined that may illustrate how neural circuitry for associative learning, neural circuitry for motivational salience, and neural circuitry for regulation of cardiovascular activity all could directly interact.

3. Background

3.1 Role of amygdala in affective arousal

Multiple studies have been done to elucidate the role of the amygdala in affective arousal and memory (McGaugh et al., 1996, Roozendaal, 1999a; Roozendaal et al., 1999b; Ferry et al., 1999, Miyashita and Williams, 2002; Curtis et al., 2002). Rats

trained on retention memory tasks showed enhanced memory if a physiologically arousing drug was injected systemically during the acquisition phase of the experiment (McGaugh et al., 1996; McGaugh et al., 1992; Roozendaal, 1999a). Lesions of the amygdala abolished the memory enhancing effect of both drugs and endogenous steroids that induce arousal. This elimination of memory enhancement was observed if the amygdala was lesioned prior to the acquisition stage, but not prior to the performance stage. This finding was valid for both general, epinephrine induced arousal, and for arousal induced by glucocorticoids during stress. Studies of humans with pre-existing brain damage limited to lesions of the amygdala showed the same lack of memory enhancement for emotionally arousing information (Cahill et al., 1995). It was concluded that the amygdala serves as a modulator of emotionally significant events, and is an important neural component in processing these events (McGaugh et al., 1992, Cahill et al., 1996). This involvement is largely dependent on the activation of the noradrenergic and the glucocorticoid neurochemical systems. The increase in NE synthesis and secretion in the amygdala during stress is largely dependent on corticotropin releasing factor, although NE levels can also become elevated in response to general physiological arousal through mechanisms independent of the stress response (Charney et al., 1995; Ferry et al., 1999c; Quirarte et al., 1997; Roozendaal, 1999a; Setlow et al., 2000).

In an inhibitory avoidance behavioral paradigm, the length of time that it took rats to enter a compartment was increased if they were previously shocked in this same compartment. Using this paradigm, it was shown that memory for a stressful experience (mild electric shock) could be influenced by both CRF and NE modifications in the BLA. (Roozendaal, 1999a; Ferry et al., 1999c) Both NE administered directly into the BLA

and CRF intercerebroventricularly (ICV) enhanced memory for a stressful experience. Infusing adrenergic receptor antagonists into the BLA immediately after the electric shock, alone or in combination with glucocorticoid receptor agonists, showed that the enhancing effect of stress on memory was mediated through beta- and alpha-adrenergic receptors in the BLA (Ferry et al., 1999a, b, c). The locus coeruleus is an important brainstem nucleus that releases NE in the amygdala and participates in modulation of stressful memories (Liang and Chiang, 1994).

Noradrenergic modulation of affective memory in the amygdala is also dependent upon NE projections from the nucleus of the solitary tract (NTS) to the BLA (Roozendaal et al., 1999b; Miyashita and Williams, 2002). In a different inhibitory avoidance task from that used by McGaugh et al. (1992), it was shown that infusion of glutamate into the NTS enhanced memory for avoiding a location associated with a mild foot shock. Blocking the beta-adrenergic receptors in BLA abolished this effect. NE concentration in the BLA, measured with microdialysis, showed significant increases following Glu infusion into NTS paired with avoidance learning (Miyashita and Williams, 2002). Concurrently it is well established that the nucleus of the solitary tract is an important structure in the control of cardiovascular regulation (Lawrence and Jarrott, 1995). The influence of NTS on the amygdala with regard to arousal and memory modulation may signify an interaction between the state of the cardiovascular response and the behavioral and learning processes mediated by the basolateral amygdala (see Figure 1).

3.2 CNS control of cardiovascular function

The CNS has a high degree of influence over blood pressure and heart rate both tonically and acutely (Longhurst, 2003). Blood pressure is tonically regulated by the sympathetic nervous system (SNS), and is sensitive to changes in SNS activity. Sympathetic control of blood pressure emanates from a nucleus on the rostral side of the medulla, called the rostral ventrolateral medulla (RVLM). Electrical stimulation of this nucleus results in transient blood pressure increases, while lesioning this structure results in negation of tonic SNS control over blood pressure and leads to a net decrease in BP. The heart also receives most of its SNS input from the RVLM. Heart rate is regulated by both sympathetic and parasympathetic nervous system activity; however it also may be influenced by more upstream neural structures.

A important regulatory system of heart is the baroreceptor reflex (see Figure 2) which couples heart rate and blood pressure through negative feedback loops. Stretch receptors located in the aorta and carotid arteries send afferent information about changes in blood pressure through the vagus nerve and glossopharyngeal nerves, respectively, to the NTS. Depending on species, the NTS discharges to either the RVLM, which projects directly to the pre-ganglionic sympathetic neurons leading to sympathoexcitation, or via the nucleus ambiguous or dorsal motor vagus to the preganglionic parasympathetic neurons leading to activation of vagal centers and enhanced parasympathetic activity. Thereby, the NTS can lead to increased HR, via excitatory discharges, or decreased HR via vagal outflow. Within the NTS, excitatory amino acids (EAA) and GABA are the main neurotransmitters mediating baroreceptor reflex activity, although neurochemical modulation of this reflex is extensive and includes vasopressin and catecholamines among other neurotransmitters (Longhurst, 2003).

CNS regulation of blood pressure also occurs indirectly, through the regulation of the levels of the neuropeptides, such as arginine vasopressin (AVP) and oxytocin. The levels of these neuropeptides in the blood are regulated by the hypothalamus and the pituitary gland. The release of AVP and oxytocin into the blood stream, and effect they have on blood pressure are via peripheral actions of these peptides, and is separate and distinct from the effect these peptides exert in the brain (de Weid, 1977). Increased levels of AVP and oxytocin cause vasoconstriction, resulting in elevated blood pressure. Although the Hypothalamic control of the cardiovascular response is affected by various neural structures, the amygdala, as part of the limbic system, has a potential role in its regulation as well. Both the basolateral amygdala and the central nucleus of the amygdala have direct projections to several hypothalamic subnuclei, including the paraventricular nucleus of the hypothalamus and the lateral hypothalamus (Price, 2003; Petrovich et al., 2005).

This important relationship between the cardiovascular brainstem nuclei, the hypothalamus, and the amygdala is further complicated by several features of the system: 1) noradrenergic arousal centrally mediated by the locus coeruleus and the NTS has a positive effect on memory in both aversive and appetitive tasks, and increases the blood pressure peripherally; 2) hypophyseal vasopressin, triggered by the hypothalamus, increases blood pressure peripherally, and also has a positive effect on memory centrally, in both appetitive and aversive tasks at certain doses (Koob et al., 1989; de Weid, 1977; de Weid et al., 1985; van Wimersma Greidanus et al., 1985); 3) the basolateral amygdala behaviorally mediates the neurochemical effects by at least (1), and most likely (2) as well; and 4) as discussed in detail below, the types of tasks which do involve mediation

by the basolateral amygdala also elicit different types of heart rate conditioning – either bradycardiac or tachycardiac in nature. Overall, there seems to be no explicit, systematic explanation which unifies all of the facts outlined above and resolves the behavioral aspects with the autonomic ones, and with the neural circuitry involved.

3.3 Influence of behavior on cardiovascular function

Cardiovascular parameters are maintained and influenced by many other factors, including elicitation of an appropriate behavioral response. The most notable behavioral influence on HR and BP is the response to a threat or stressful stimulus. A "fight or flight" response includes activation of the SNS along with behavioral, endocrine, and visceral activation. The cardiovascular part of this response, being sympathetic activation, is mediated primarily through the RVLM. In addition, the NTS control of baroreceptor reflex is inhibited during this increase in BP/HR. Acoustic and airpuff startle paradigms are examples where a mild but stressful stimulus elicits conditioned cardiovascular responses (Young and Leaton, 1996; Taylor et al., 1994; Abdeen et al., 1995). In naïve animals, the airpuff stimulus (with attendant tonal qualities) causes tonic deceleration of heart rate, also known as conditioned bradycardia, that results from parasympathetic activation. This response, the cardiovascular correlate of the orienting response, rapidly habituates over time. The acoustic component of the airpuff, as well as the acoustic component of acoustic startle stimuli, causes a phasic acceleration of heart rate that is developed rapidly over timeand indicates increases in sympathetic nervous system activity.

Another way that behavior influences cardiovascular response is evident in classical conditioning of bradycardiac response to the neutral stimulus paired with an aversive US (Frysztak and Neafsey, 1986; LeDoux, 1993). A number of studies with fear conditioning to context paradigm demonstrated a pervasive conditioned increase in blood pressure as well as conditioned bradycardia to a negative environment (Carrive, 2000; Carrive, 2002; Faneslow, 1980). The author's conclusion was that the cardiovascular response in the case of aversive contextual conditioning is entirely mediated by the autonomic nervous system (Carrive, 2002). Although most of the experiments with conditioned bradycardia involve aversive conditioning paradigms (Powell et al., 1997, Young and Leaton, 1996), some appetitive conditioning paradigms have been employed with cardiovascular response as well (Hunt and Campbell, 1997; Kostarczyk and Fonberg, 1982).

In one study, dogs were trained to perform an instrumental action (foreleg on a food tray) upon hearing a CS (a tone), in order to receive a US (petting on head and back) (Kostarczyk and Fonberg, 1982). The heart rate was decelerated in response to the US, and accelerated at the offset of the US. Following training, heart rate initially decelerated to the CS, and then accelerated at the onset of the instrumental response. In another appetitive conditioning study, rats were classically conditioned with light (CS) – food (US) pairings (Hunt and Campbell, 1997). Following training, when conditioned orienting (CR) was achieved to the light, heart rate and blood pressure were measured. Heart rate deceleration was observed during CS presentation, in parallel to the aversive conditioning studies, however, there was no heart rate increase to the US that sometimes follows bradycardia in the aversive conditioning paradigms (Young and Leaton, 1996).

Both aversive and appetitive conditioning paradigms have elicited changes in cardiovascular response. The amygdala plays an extensive role, discussed above, in these types of associative learning tasks. The following section discusses some of the influences on the cardiovascular response that are specific to the amygdala.

3.4 Role of the amygdala in cardiovascular response

There is a large volume of research that specifically implicates amygdalar processing in regulating blood pressure and heart rate. Most of this literature implicates conditioned bradycardia response (Young and Leaton, 1996; Powell et al., 1997; Kapp et al., 1979; Kapp et al., 1982). Some of the earlier studies demonstrated that lesions of the CeA prevent acquisition of conditioned bradycardia in the rabbit (Kapp et al., 1979). Lesions of the CeA also attenuate changes in heart rate evoked during the acoustic startle paradigm (Young and Leaton, 1996). The CeA has extensive GABAergic projections to the NTS, as well as putative glutamatergic projections to the RVLM, both of which are in direct control of heart rate and blood pressure modulation (Kapp et al., 1982; Zhang et al., 1986; Saha et al., 2000; Saha, 2005). Electrical stimulation of the CeA in the rabbit leads to cardiovascular and autonomic conditioning representative of fear and anxiety responses (Kapp et al., 1982).

The projections of the central nucleus of the amygdala to NTS as well as the nucleus ambiguus and the dorsal motor nucleus of the vagus are hypothesized to play a significant role in this cardiovascular regulation (Powell et al., 1997). Bilateral lesions of the basolateral complex of the amygdala also completely prevents bradycardia conditioning in the rabbit in the aversive classical conditioning paradigm (Powell et al.,

1997). It has been hypothesized that interconnectivity of BLA with the prefrontal cortex (PFC) plays a large role in this phenomenon, since lesions of the PFC, which has projections to the autonomic nuclei in the brainstem, also abolish conditioned bradycardia (Buchanan and Powell, 1982). This hypothesis was proven wrong in an elegant crossed unilateral lesion study (Powell et al., 1997), so the circuitry underlying the effect of BLA on cardiovascular response remains largely unresolved. Other studies have looked at the influence of the intact BLA on cardiovascular response by infusing various agents directly into BLA sans any behavioral manipulation (Soltis et al., 1997; Shekhar et al., 1999; Sajdyk and Shekhar, 1997). Specifically, GABAa mediated processes in the amygdala have been implicated in changes in heart rate and blood pressure (Sajdyk and Shekhar, 1997). Infusing a GABAa antagonist, bicuculline methiodide (BMI), directly into BLA increases heart rate and blood pressure in a dose dependent manner in awake behaving rats (Soltis et al., 1997). Infusion of glutamate receptor blockers into BLA has no significant effect on CV measurements in isolation (Sajdyk and Shekhar, 1997), however infusing NMDA and AMPA antagonists together with BMI blocks the BMI induced HR increase in a dose dependent manner (Soltis et al., 1997). Conversely, infusion of glutamate agonists increases HR in a dose dependent manner, which implicates that excitatory amino acids regulate GABA cells in the BLA with respect to cardiovascular modulation (Soltis et al., 1997; Shekhar et al., 1999).

The basolateral amygdala may also control cardiovascular response indirectly, through the influence that the BLA and the CeA exert on the hypothalamus (Petrovich et al., 2005; Price, 2003), and the hypothalamic projections to the pituitary and the

hypophyseal portal blood system where neuropeptides, such as vasopressin and oxytocin, both potent vasoconstrictors, are released.

The findings described above clearly indicate that the amygdala plays a significant role in the regulation of some aspects of the cardiovascular response, specifically heart rate modulation, either with or without the involvement of behavioral conditioning. Furthermore, BLA and CeA are both independently important for the maintenance of this influence (Powell et al., 1997).

The goal of this experiment is to provide initial data for further investigation of the correlation between behavioral changes on the instrumental version of the conditioned preference task and cardiovascular changes in heart rate and blood pressure. This investigation will aim to clarify the role of the basolateral amygdala in relation to the cardiovascular response in this appetitive task.

Outline of the paradigm: A group of rats received BLA targeted permanent cannulae implants and, following recovery, were then acclimated to the baseline "flower pot" environment and to the contingent maze environment. Then all rats underwent arterial catheterization, and, following a recovery period, heart rate and blood pressure measurements were collected during context switch from non-contingent appetitive baseline behavior to the contingent appetitive learning behavior and back. The animals that sustained transient BLA inactivation during CPP training failed to acquire place preference, while rats with intact BLA during CPP training demonstrated conditioned preference for the reinforced arm of the maze. There was also a significant relationship between the cardiovascular response and the contextual switch between the baseline and the CPP learning environment.

4. Materials and Methods

Long-Evans hooded rats (Charles River, Raleigh NC), n=6, began behavioral testing. Water was available *ad libitum*, however, food was restricted to maintain body weight at 90% of their free-feeding weight for motivation by a food reward.

4.1 Surgical Procedures - BLA inactivations

Methods are as described in detail in Hatfield and Gallagher, 1995. AP-5 infusion was employed to reversibly inactivate the basolateral amygdalae. Briefly, following the surgery preparation described in Exp. 1, rats were implanted bilaterally with 26 ga stainless steel cannulae (Plastics One, USA). Each cannula was stereotaxically lowered into the brain to position it just above the BLA (6.4 mm from the skull, at 2.8 mm posterior to bregma and 5 mm lateral from the midline) and was fixed in place by attaching it to two anchor screws in the skull with dental cement. Removable stainless steel dummy cannulae (Plastics One) were inserted in the cannulae to keep them patent. For the inactivation, 2.5µg/side of AP-5 dissolved in artificial CSF (aCSF) was infused through 33 ga beveled infusion cannulae connected by an infusion system (Plastics One) to a 10 µl Hamilton syringe mounted in a microinfusion stereotaxic attachment.

4.2 Arterial Catheterization

Following behavioral pre-training on the task described above, all rats underwent catheterization of the left femoral artery for the assessment of the blood pressure and heart rate changes during this task. Catheters were constructed by heat fusing PE-10 and

PE-50 tubing together and checked for leaks. Under Isoflurane gas anesthesia, the femoral artery was located within the femoral triangle and exposed for catheter insertion. The catheter, filled with sterile heparinized saline, was inserted into the femoral artery and advanced rostrally 4.5 cm into the abdominal aorta positioning it just below the renal arteries. Following securing the catheter in place, it was filled with sterile heparinized saline (30% heparin), plugged, tunneled under the skin, and exteriorized at the nape of the neck. Following surgery, animals were housed 1 per cage on a 12-hour light-dark cycle. Food and water were available ad libitum and 3 days' recovery was allowed before testing. Catheters were flushed daily during recovery with heparinized saline to prevent clotting and to maximize the time that the catheter can be used effectively. After the beginning of the experiment, catheters were flushed with heparnized saline every other day.

For cardiovascular measurements during the behavioral testing, the exteriorized catheter was attached to a saline filled length of PE-50 tubing (50cm), which in turn was attached to a pressure transducer and blood pressure analyzer (BPA, Digi- Med, Micromed Assoc., Louisville, KY, USA). This permitted both blood pressure (BP) and heart rate (HR) to be recorded as digitized 0.5 second averages, as well as a continuous waveform for future analysis. BP was recorded for 10 minutes before the start of the behavioral task to establish a "before" baseline measurement. Considering that rats ingested sweet cereal during the behavioral task, BP measurement continued for 10 minutes after the end of the task to establish an "after" baseline. The data was stored and extracted using Digi-Med System Integrator Model 200/1 software.

4.3 Behavioral Apparatus and Procedure

The platform used for all of the CV behavioral measures is the same as described in Chapter 1. A three arm maze constructed out of red transparent plexiglass was used for both the acquisition and the retention of the CPP paradigm in this experiment. The maze was placed on top of the platform, which was surrounded by a dark curtain. Limited distal and no proximal cues were used in order to bias the behavioral learning toward amygdalar activation. The maze consisted of three different compartments, each measuring approximately 15 X 25cm. A central "start" area was defined at the entrance to the maze, measuring approximately 25cm by 20cm. All of the maze compartments, as well as the "start" or "neutral" area, were 60 cm tall. Each of the compartments, left, central, and right, could be closed off by sliding plexiglass barriers. A sweet cereal (Honey Nut Cheerios, Kellogg, Inc.) was used as reinforcement in this experiment. The following CPP methods were loosely adapted from White and McDonald, 1993, and from Tzschentke, 1998.

During the pre-training for this experiment, rats were accomodated to the experimental room by being allowed to explore the maze and the open-field platform with uniformly distributed pieces of Cheerios. . The rats were also accomodated to the flower pot with bedding and pieces of Cheerios, which served as the baseline environment. During the training trial of this experiment, the maze was placed on the platform, the central compartment of the maze closed off, and a pile (approx. 30g) of Honey Nut Cheerios was placed in one of the open compartments, thus making this particular compartment the "+ arm" of the maze.

During the training day, the rats received a targeted BLA infusion of either AP-5 in aCSF or aCSF alone in a different room. The rats were then brought into the experimental room and the externalized arterial catheters were hooked up to the Blood Pressure Acquisition system (BPA). Baseline heartrate (HR) and blood pressure (BP) measurements were taken for 5 to 10 minutes, in the flower pot filled with bedding with the sweet cereal freely available, and the rats then placed in the "start" portion of the maze. The rats were allowed to explore the maze for a time period of 5-10 minutes. After the maze exploration, the rats were transferred back into the flower pot and the post-baseline HR and BP were recorded for 5 to 10 minutes.

During the test day, 48 hours later, the maze was placed on the platform, in an identical location to training day, with all compartments open and available for exploration. The central compartment now served as a novel arm. The rats were brought into the experimental room, hooked up to the BPA system, and, following the baseline BP/HR recording as described above, placed in the "start" location of the maze. The rats were then allowed to freely explore the maze for a period of 5-10 minutes. Again, following the maze exploration, the rats were put back into the baseline environment, and the post-baseline measurements were taken for 5 to 10 minutes before the rats were transferred back to their home cages.

Following a 4-5 day break, the experimental procedure was repeated with either an AP-5 or an aCSF infusion and reinforcement placed in a different arm of the maze, using a balanced design.

All behaviors were recorded with a video camera, digitized into MPEG format, and analyzed for time spent in the baseline environment, and in all three arms of the maze.

4.4 Statistical Analysis

All data were analyzed using JMP 4.0.2 by the SAS institute. The blood pressure and heart rate data were superimposed on the coded and time stamped description of the rat behavior from the digitized video. The cardiovascular response data were pooled and averaged for pre-baseline, maze, and post-baseline behaviors. Single point averages of the blood pressure data were subsequently used for statistical analyses. Because the rats received qualitatively different treatment with respect to the infusions on the training day of CPP and on the test day of CPP, the data for the CPP training and the CPP test days were analyzed separately. For each CPP day (n=4), a two way repeated measures ANOVA (3 X 2) was performed on the blood pressure data, – the within variables were the context (pre-baseline, maze, and post-baseline (3)), and the condition (AP5(n=4)/vehicle(n=2) or postAP5/post-vehicle(same n)). Step down ANOVAs and post-hoc comparisons were performed on data variables with significant main effects and interactions.

5. Results

The transfer of rats between the non-contingent appetitive context and the contingent appetitive context had a significant effect on the levels of mean arterial blood

pressure, both during the CPP training day (main effect of context: $F(2,5)=12.84$, $p=0.01$) and during the CPP test day (main effect of context: $F(2,5)=12.62$, $p=0.011$).

Step down analyses showed that the switch of context from the pre-baseline behavior to the maze behavior significantly lowered the blood pressure in all animals included in the study ($F(1,16)=49.45$, $p<0.0001$; see Figure 4 and Figure 5). Switching the rats back into the post-baseline context after the maze resulted in significant increases in the blood pressure in all animals ($F(1,16)=33.39$, $p<0.0001$; see Figure 4 and Figure 5). These blood pressure changes were stable and unchanging for both the training day and the testing day on the Conditioned Place Preference paradigm, as measured by a one way repeated measures ANOVA (main effect of CPP trial: $F(1,7)=2.35$, $p=0.13$).

The integrity of the basolateral amygdala had a marginal effect on the robust decrease in blood pressure (main effect of AP5: $F(1,17)=3.83$, $p=0.062$; see Figure 5A, 5C). The decreases in blood pressure during maze behavior were still present during the AP5 infusion into BLA; however, the changes from pre-baseline, and back to post-baseline BP levels during AP5 inactivation on the CPP training day were less pronounced than during either vehicle infusion or during the CPP test day measures (see Figure 5A).

The heart rate did not respond to the switch in context in the same way, in fact, the changes in heart rate were negligible and highly variable in all animals and all measured instances. Neither blood pressure nor heart rate recorded during baseline differed depending on the temporary inactivation of the basolateral amygdala. Both the pre-baseline and post-baseline blood pressure was the same during the training day of the CPP behavior both with and without BLA inactivation ($F(1,16)=1.57$, $p=0.228$; see Figure 4 and Figure 5).

The differences in individual animals' baseline heart rate and blood pressure was too great to attempt to average the values directly across different rats, or across different days in the same rats. However, when the the difference in averaged BP was computed for the switches between contexts, the changes in blood pressure between contexts were uniformely decreased to maze, and increased back to post-baseline (Posthoc, CPP training, main effect of switch: $F(1,3)=74.44$, $p=0.0033$; Posthoc, CPP test, main effect of switch: $F(1,3)=30.14$, $p=0.012$; see Figure 3A, 3B). Also, although the baseline BP values were highly variable, the trends in blood pressure rise and fall during different context behaviors were consistent in all animals and instances observed (see Figure 4).

The behavioral results following CPP conditioning were in accordance with those attained through similar methods by White and McDonald, 1993. A two way repeated measures (2 X 3) ANOVA analysis (AP5 or aCSF infusion by maze compartment (Arm +, Arm-, Neutral))showed a significant interaction between the BLA inactivation and the time spent in the maze arms ($F(2, 2) = 23.3$, $p=0.0149$). Step down analyses demonstrated that after place preference training rats with intact BLA tended to spend significantly more time in the previously reinforced, versus the un-reinforced arm of the maze (vehicle rats contrast, maze compartment: $F (1, 3)=16.66$, $p=0.026$; see Figure 6). Rats with the transiently inactivated basolateral amygdala did not spend significantly more time in either arm of the maze (AP5 rats contrast, maze compartment: $F (1, 3) = 1.4$, $p=0.32$; see Figure 6).

The circuitry underlying these behavioral and cardiovascular responses includes learning, motivational, and physiological neural components (see Figure 7). It is

plausible that all of the circuitries represented play a role in this behavioral and accompanying cardiovascular response to place preference learning.

6. Discussion

The neural circuitry that underlies autonomic – behavioral coupling has the amygdala, including both basolateral and central amygdala at its heart, by virtue of bidirectional connectivity with a) OFC, which participates in object – valence associations and informs behavioral output in affective associative learning (Schoenbaum et al., 1998; Schoenbaum et al., 1999; Gallagher and Chiba, 1996; Baxter and Murray, 2000; Chiba et al., 2002), b) NTS, which participates in cardiovascular regulation (Lawrence and Jarrott, 1995), as well as influencing affective memory (Roozendaal et al., 1999; Miyashita and Williams, 2002), c) CeA and the extended amygdala, which participate in the processing of affect, reward, and sensorimotor integration among other functions, and d) the hypothalamus, which exerts direct influence on the release of the hypophyseal peptides and the resultant changes in cardiovascular response (Weid, 1977; Sahgal, 1984; Koob et al., 1989). This extensive interconnected network of cortical and subcortical structures has been implicated in other types of rewarding and aversive processing, such as stress response and drug addiction (Koob and Le Moal, 2001; Everitt et al., 2000).

These results may shed light on the role of the amygdala in integrating physiological and neural signals in order to modulate behavioral output. The variations in cardiovascular response that accompany the context changes and the behavioral output in this task may directly participate in the process of associative learning by a dual

process of a) facilitating learning of contingencies directly, and by b) stimulating the limbic structures involved in context conditioning through feedback about the level of the physiological arousal of the animal. Conversely, the process of a maze arm acquiring positive association may have a physiological correlate, such as a change in cardiovascular response, that coincides with, or even preceeds the change in behavioral output toward this maze arm.

The results of this experiment indicate that there is a definite relationship between changes in cardiovascular response and context dependent differences in contingencies. The effect of the switch from the non-contingent baseline context into the contingency of the maze environment elicited a robust decrease in blood pressure.

However, these results are at odds with numerous studies on aversive conditioning and a few studies on appetitive conditioning that show, conversely, an increase in blood pressure accompanying a switch to a contingent context (Carrive, 2000; Iwata and LeDoux, 1988; Ettenberg et al., 1983). The origins of these differences may involve several explanations. First, it should be recognized that there are numerous differences between the previous studies and the present experimental approach; there are also postulations of a U-curve relationship between the levels of arousal and efficacy of learning and behavioral performance; there is also a qualitative difference between appetitively and aversively motivated learning; and there is a qualitative neural difference between instrumental and classical conditioning (Koob et al., 1989; Killcross et al., 1998; White, 1989). The interpretation of our results are further complicated by the known relationship between BLA, norepinephrine, arousal, the NTS, and affective memory

(McGaugh et al., 1996, Roozendaal, 1999a; Roozendaal et al., 1999b; Ferry et al., 1999c, Miyashita and Williams, 2002; Curtis et al., 2002).

The potential role of the BLA becomes clearer if the relationship between its inactivation and the blood pressure levels in the maze is examined more carefully. During the BLA inactivation, the fall of BP levels in the maze is less pronounced, while in every other instance, the fall of BP in the maze is remarkably similar (see Figure 3A, 3B). This lack of a pronounced decrease in blood pressure that we observed with an inactivated BLA is, at this point, a trend, as opposed to a statistically significant result. If this result is replicated with more animals, then the following might explain the relationship between the BLA and the cardiovascular response during affective learning. It is possible that during this type of learning, the BLA is indirectly involved in BP regulation through the connections between the CeA and NTS, as well as between the BLA and the CeA and the hypothalamus (Petrovich et al., 2005; Price et al., 2003; Miyashita and Williams, 2002). Activation of the CeA inputs into the NTS, which are GABAergic in nature (Saha, 2005; Saha et al., 2000), would cause the baroreceptive reflex to cease updating the heart rate properly, resulting in negligible changes in heart rate (Sato et al., 2003). There exists evidence that the unit activity in the paraventricular nucleus of the hypothalamus is regulated by the noradrenergic brainstem inputs during emotional learning (Nakamura et al., 1992). This neural activity is correlated with blood pressure regulation during both aversive and appetitive cue learning (Nakamura et al., 1992). Activation of the amygdalar-hypothalamic efferents might regulate this hypothalamic influence on blood pressure during the switching of behavioral context.

On the other hand, inactivation of the BLA could interfere with regulation of both the baroreceptor reflex and the hypothalamic regulation, leading to aberrant influence on both of these mechanisms, and resulting in altered BP levels during contingency learning.

This line of reasoning plays directly into an old and well established hypothesis on the role of AVP and the AVP pressor properties in learning behavior, explored extensively in a number of aversive as well as appetitive conditioning paradigms (Weid, 1977; Sahgal, 1984; Ettenberg et al., 1983; Koob et al., 1989). The variability between our results and the predictions of these research studies could be explained by the differences in pre-training, training, and testing procedures, as well as the baseline comparison conditions. Unlike the previous studies, our baseline comparison included a large amount of appetitive reinforcement, delivered in a non-contingent manner, which could account for the higher baseline blood pressure if we were to compare it to the resting blood pressure in the home cage. There is evidence in literature that supports this conjecture: in a context shift study with a use of a non-contingent appetitive context, an increase in mean arterial pressure accompanied the shift to this context from a food deprived baseline (Braesicke et al., 2005). A neurotoxic lesion of an entire amygdala had no effect on blood pressure in the non-contingent appetitive environment, but interfered with the autonomic arousal during anticipation (Braesicke et al., 2005) This is a possible avenue for future experiments.

The CPP paradigm is mediated by many different structures besides the BLA, such as the hippocampal formation, caudate and putamen, and the prefrontal cortex (Chai and White, 2004). Conversely, the blood pressure levels are regulated by many autonomic structures besides the hypothalamus. This may explain why the blood

pressure changes due to context switching are robust and present on both the training and the test days of the CPP paradigm as well as for both the intact and the inactivated BLA.

In the conditioned bradycardia experiments, bilateral BLA lesions resulted in attenuation of conditioned bradycardia in response to CS presentation in rabbits (Powell et al., 1997). In sham lesioned rats, heart rate progressively decelerated in response to the onset of the conditioned stimulus (auditory tone), over the course of successive trials in classically conditioned bradycardia (Powell et al., 1997). In an appetitive instrumental paradigm, heart rate in dogs decelerated during the administration of petting reward (US), and then accelerated after the cessation of reward (Kostarczyk and Fonberg, 1982). In the same paradigm, at the onset of CS (tone), heart rate accelerated, and then decelerated during US administration. These findings are also consistent with the heart rate variability and inconsistent effects during this task.

In sham lesioned rats, heart rate initially decelerated in response to unconditioned aversive stimulus (electric shock), and then accelerated in both classically conditioned bradycardia (Powell et al., 1997), and in the acoustic startle paradigm (Young and Leaton, 1996). In an appetitive classical conditioning paradigm, heart rate decelerated in response to a food reward, without acceleration thereafter (Hunt and Campbell, 1997).

Due to the large variability in HR/BP baselines between individual animals it may not be possible to reliably assess the quality and extent of the difference between inactivated and vehicle infused animals. In the present case, it will be beneficial to replicate our findings by measuring cardiovascular response using radiotelemetry.

A novel interpretation for the activity of this circuitry is herein proposed by uniting the theories of amygdalar involvement in associative learning (Baxter and

Murray, 2002; Gallagher and Chiba, 1996), with the hypotheses about amygdalar involvement in regulating cardiovascular and behavioral output (Powell et al., 1997; Gallagher et al., 1972). We have also integrated some of what is currently known about the relationship between affective conditioning and hypophyseal peptides together with what is known about the role of the BLA in affective learning and arousal.

The future directions for this line of work could involve examining more closely the role of the BLA in modulation of the hypophyseal peptide effect on learning, as well as exploring the role of the central nucleus of the amygdala as the intermediate structure in affective learning and control of the physiological arousal.

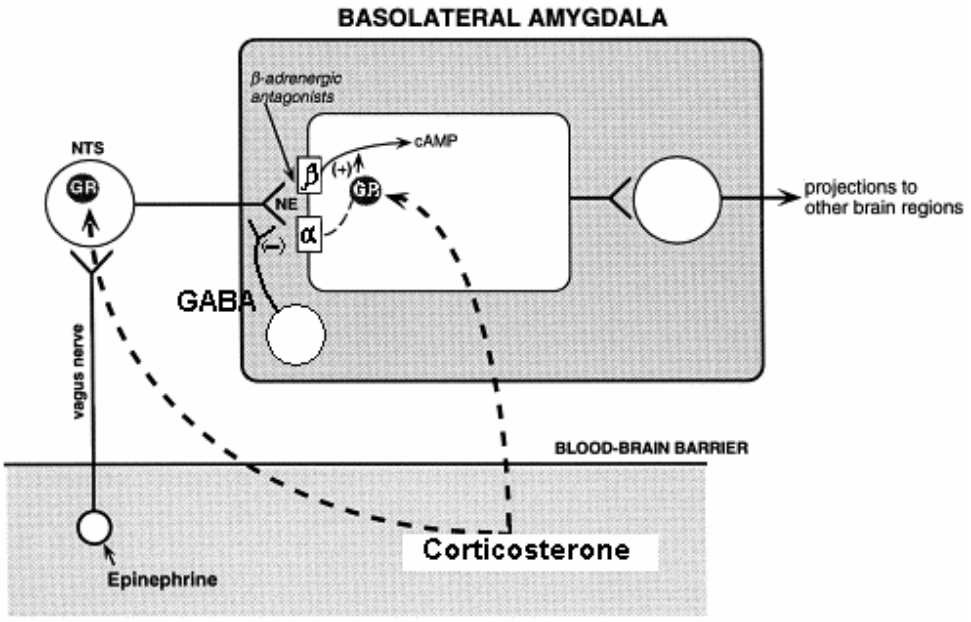


Figure 5.1 Neural circuitry underlying modulation of affective memory by BLA. Adapted from McGaugh et al., 2000.

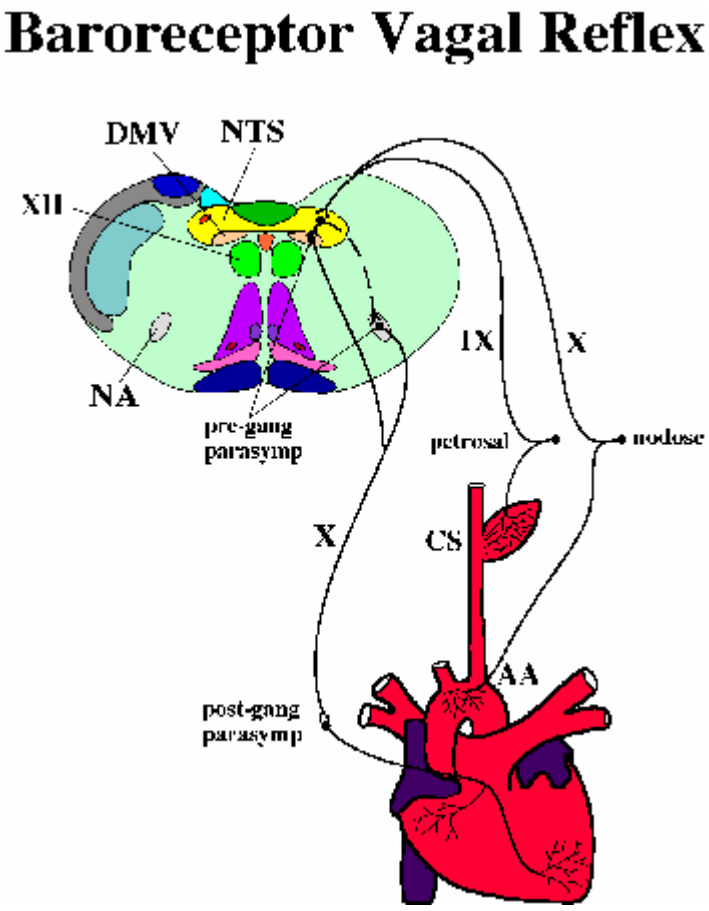
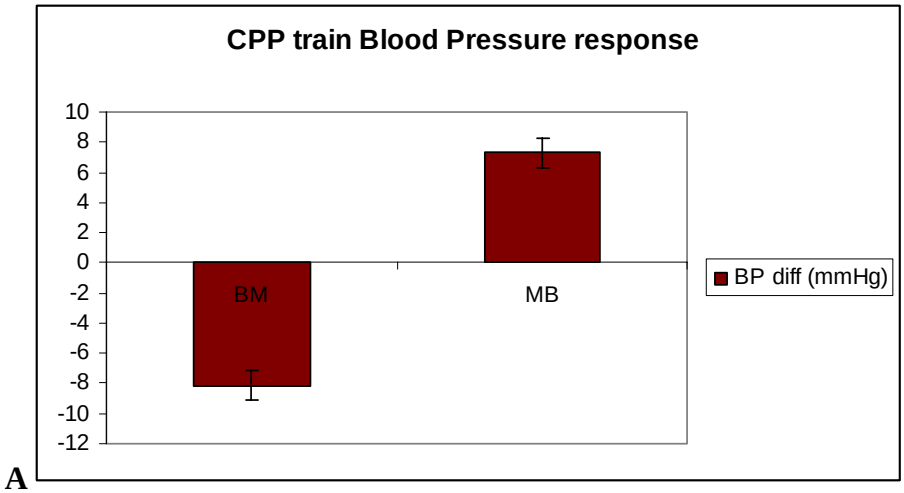
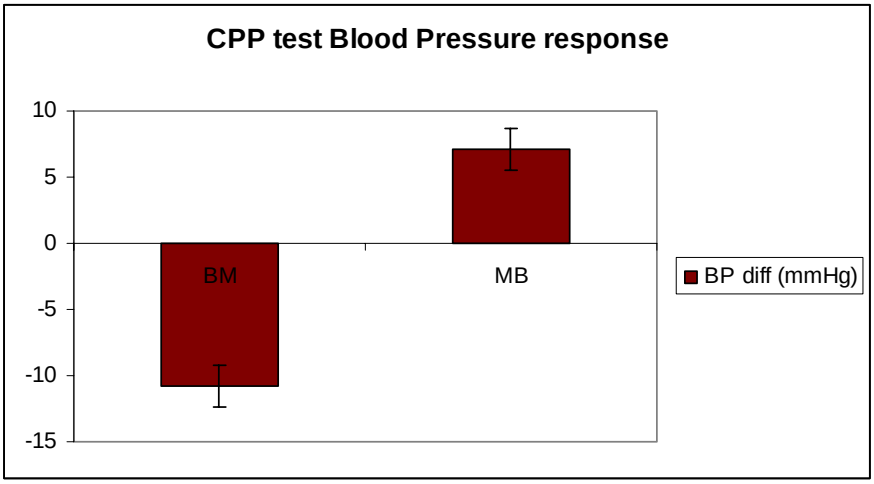


Figure 5.2 Visual description of the structures and pathways involved in baroreceptor reflex mediation. AA - aortic arch; DMV - Dorsal Motor nucleus of the vagus; CS – carotid sinus; NA – nucleus ambiguus; NTS – nucleus of the solitary tract; IX, X, XII – cranial nerves. Adapted from Rybak et al., 2002.



A



B

Figure 5.3 The differences in blood pressure during contextual context switches (mmHg; Y-axis), pooled and averaged over 10 complete CPP trials (both AP5 and vehicle trials included; *n*=4), (Pre-Baseline to Maze change and Maze to Post-Baseline change; X-axis). **(A)** The relative difference in blood pressure (mmHg) from Pre-Baseline to Maze (BM), and from Maze to Post-Baseline (MB) on the training day of the Conditioned Place Preference paradigm **(B)** same as (A) for the test day of the CPP paradigm, 48 hours later.

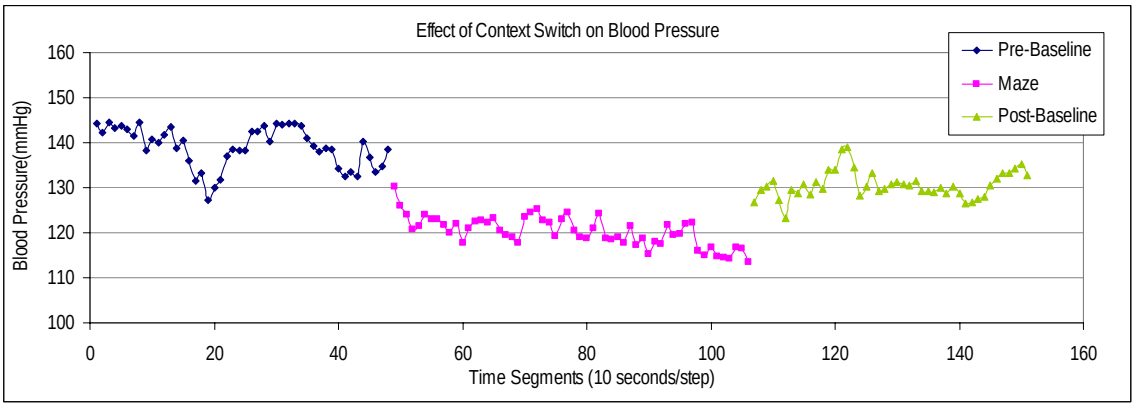


Figure 5.4 Representative trend in blood pressure change over time (mmHg; Y-axis) Rise and fall of BP during different context behaviors over time (average BP over 10 second time segments; X-axis). Blood pressure is reliably lower during maze behavior compared to pre- and post-baseline behavior. Furthermore, there is a downward slope trend to blood pressure while on maze, as opposed to a relatively stable and unchanging blood pressure during pre- and post baseline.

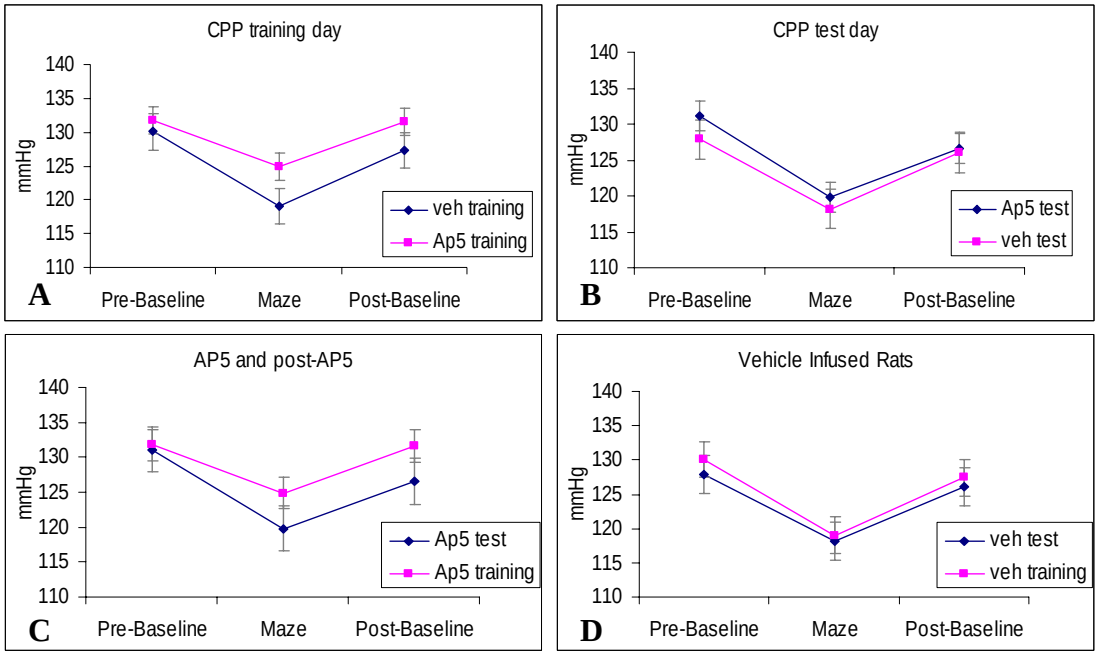


Figure 5.5 Averaged BP data for all rats in contingent and non-contingent contexts: These graphs depict single point averages of mean arterial blood pressure (MAP mmHg; Y-axis), pooled and averaged for the pre-baseline, maze and post-baseline environments (X-axis) across all rats in the following categories. **(A)** This graph contains blood pressure points pooled and averaged across AP5 infused (*n*=4) and the vehicle infused (*n*=2) rats for the training day of CPP. **(B)** This graph contains pooled and averaged blood pressure points for the rats in (A), but 48 hours post-infusions, during the testing day of CPP. **(C)** This graph contains blood pressure points pooled and averaged across the rats with the BLA inactivation during both CPP training (*n*=4) and 48 hours post-infusion on CPP test day (*n*=4). **(D)** This graph contains averaged blood pressure points averaged across the rats with the intact BLA during both CPP training (*n*=2) and 48 hours post-infusion (of vehicle) on CPP test day (*n*=2).

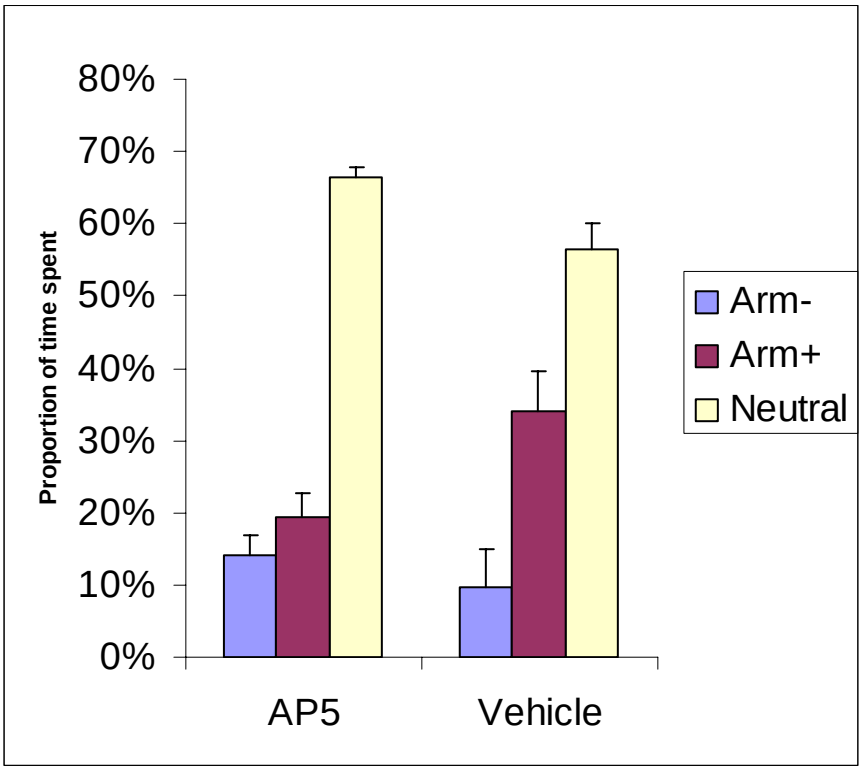


Figure 5.6 Behavioral results of the conditioned place preference experiment in a three-arm maze The graph shows the percentage proportion of time (Y-axis) spent in different maze compartments on the test day of the CPP paradigm for previously Ap5 infused or vehicle infused animals (X-axis). AP5 means that the animals tested had their basolateral amygdalae inactivated bilaterally 48 hours previously (n=4). Vehicle means that the animals tested had aCSF infused into their BLA bilaterally 48 hours previously (n=3).

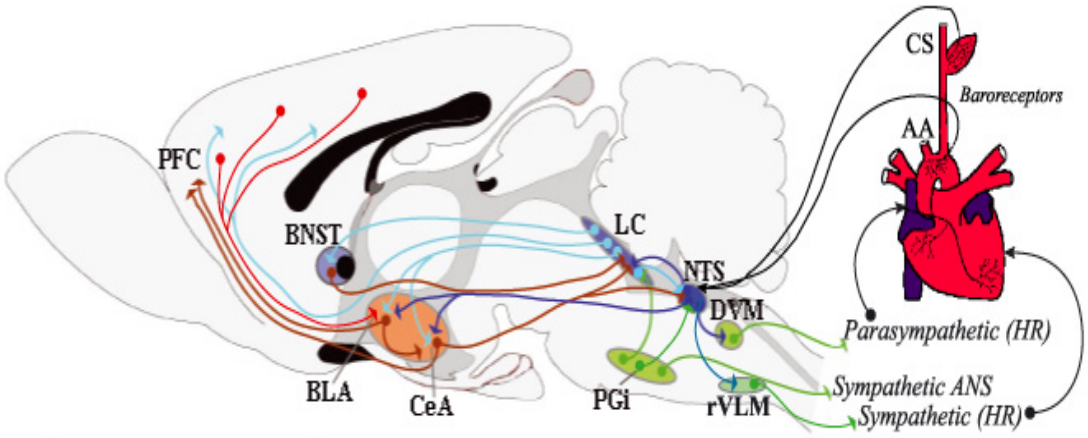


Figure 5.7 The circuitry underlying the behavioral and cardiovascular responses discussed in this Chapter include learning, motivational, and physiological neural connections. This figure depicts the interconnectivity between the amygdala components (BLA and CeA) and the cognitive (PFC – Prefrontal cortex), motivational (BNST – Bed Nucleus of Stria Terminalis), and physiological (LC – locus coeruleus, NTS – nucleus of the solitary tract) loci that participate in the elicited physilogical and behavioral effects. There are numerous structures (some not shown) also associated with these loci that together coordinate and exert these effects. (Abbreviations: DVM – dorsal motor nucleus of the vagus, PGi – nucleus peri-gigantocellularis, rVLM – rostral ventro lateral medulla, AA – aortic arch, CS – carotid, ANS – autonomic nervous system). *Schematically drawn by I.Y. Merzlyak*

7. References

Abdeen, O. A., Taylor, B. K., Youngblood, K. L., & Printz, M. P. (1995). Peripheral beta adrenergic blockade modifies airpuff startle-induced heart rate responses. J Pharmacol Exp Ther, 272(1), 282-289.

Alemayehu, A., Breen, L., Krenova, D., & Printz, M. P. (2002). Reciprocal rat chromosome 2 congenic strains reveal contrasting blood pressure and heart rate QTL. Physiol Genomics, 10(3), 199-210.

Balleine, B.W., Leibeskind, J.C. and Dickinson, A.: Effects of cell body lesions of the Basolateral Amygdala on instrumental conditioning. Soc. Neuroscience Abstract 1997, 23, 786.

Braesicke K, Parkinson JA, Reekie Y, Man MS, Hopewell L, Pears A, Crofts H, Schnell CR, Roberts AC. Autonomic arousal in an appetitive context in primates: a behavioural and neural analysis. Eur J Neurosci. 2005 Mar;21(6):1733-40.

Buchanan, S. L., & Powell, D. A. (1982). Cingulate damage attenuates conditioned bradycardia. Neurosci Lett, 29(3), 261-268.

Buchanan, S. L., & Thompson, R. H. (1994). Neuronal activity in the midline thalamic nuclei during Pavlovian heart rate conditioning. Brain Res Bull, 35(3), 237-240.

Carrive P. (2000). Conditioned fear to environmental context: cardiovascular and behavioral components in the rat. Brain Res. Mar 10;858(2):440-5.

Chai SC, White NM. Effects of fimbria-fornix, hippocampus, and amygdala lesions on discrimination between proximal locations.

Curtis, A. L., Bello, N. T., Connolly, K. R., & Valentino, R. J. (2002). Corticotropin-releasing factor neurones of the central nucleus of the amygdala mediate locus

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.

de Wied D, Gaffori O, van Ree JM, de Jong W. Central target for the behavioural effects of vasopressin neuropeptides. Nature. 1984 Mar 15-21;308(5956):276-8.

de Wied D. Behavioral effects of neuropeptides related to ACTH, MSH, and betaLPH. Ann N Y Acad Sci. 1977 Oct 28;297:263-74.

Everitt, B. J., Cador, M., & Robbins, T. W. (1989). Interactions between the amygdala

and ventral striatum in stimulus-reward associations: studies using a second-order schedule of sexual reinforcement. Neuroscience, 30(1), 63-75.

Everitt, B. J., Morris, K. A., O'Brien, A., & Robbins, T. W. (1991). The basolateral amygdala-ventral striatal system and conditioned place preference: further evidence of limbic-striatal interactions underlying reward-related processes. Neuroscience, 42(1), 1-18.

Ferry, B., Roozendaal, B., & McGaugh, J. L. (1999). Role of norepinephrine in mediating stress hormone regulation of long-term memory storage: a critical involvement of the amygdala. Biol Psychiatry, 46(9), 1140-1152.

Frysztak, R. J., & Neafsey, E. J. (1994). The effect of medial frontal cortex lesions on cardiovascular conditioned emotional responses in the rat. Brain Res, 643(1-2), 181-193.

Gallagher, M., & Chiba, A. A. (1996). The amygdala and emotion. Curr Opin Neurobiol, 6(2), 221-227.

Gallagher, M., Kapp, B. S., & Pascoe, J. P. (1982). Enkephalin analogue effects in the amygdala central nucleus on conditioned heart rate. Pharmacol Biochem Behav, 17(2), 217-222.

Hatfield, T., Han, J. S., Conley, M., Gallagher, M., & Holland, P. (1996). Neurotoxic lesions of basolateral, but not central, amygdala interfere with Pavlovian second-order conditioning and reinforcer devaluation effects. J Neurosci, 16(16), 5256-5265.

Heimer, L., Alheid, G. F., de Olmos, J. S., Groenewegen, H. J., Haber, S. N., Harlan, R. E., & Zahm, D. S. (1997). The accumbens: beyond the core-shell dichotomy. J Neuropsychiatry Clin Neurosci, 9(3), 354-381.

Hiroi, N., & White, N. M. (1991). The lateral nucleus of the amygdala mediates expression of the amphetamine-produced conditioned place preference. J Neurosci, 11(7), 2107-2116.

Hunt, P. S., & Campbell, B. A. (1997). Autonomic and behavioral correlates of appetitive conditioning in rats. Behav Neurosci, 111(3), 494-502.

Iwata J, LeDoux JE. Dissociation of associative and nonassociative concomitants of classical fear conditioning in the freely behaving rat. Behav Neurosci. 1988 Feb;102(1):66-76

Kapp, B. S., Frysinger, R. C., Gallagher, M., & Haselton, J. R. (1979). Amygdala central nucleus lesions: effect on heart rate conditioning in the rabbit. Physiol Behav,

23(6), 1109-1117.

Killcross, S., Robbins, T. W., & Everitt, B. J. (1997). Different types of fear-conditioned behaviour mediated by separate nuclei within amygdala. Nature, 388(6640), 377-380.

Kostarczyk, E., & Fonberg, E. (1982). Heart rate mechanisms in instrumental conditioning reinforced by petting in dogs. Physiol Behav, 28(1), 27-30.

Lang, P. J. (1995). The emotion probe. Studies of motivation and attention. Am Psychol, 50(5), 372-385.

Lawrence, A. J., & Jarrott, B. (1996). Neurochemical modulation of cardiovascular control in the nucleus tractus solitarius. Prog Neurobiol, 48(1), 21-53.

LeDoux, J. E., Iwata, J., Pearl, D., & Reis, D. J. (1986). Disruption of auditory but not visual learning by destruction of intrinsic neurons in the rat medial geniculate body. Brain Res, 371(2), 395-399.

Lewis, S. J., Verberne, A. J., Robinson, T. G., Jarrott, B., Louis, W. J., & Beart, P. M. (1989). Excitotoxin-induced lesions of the central but not basolateral nucleus of the amygdala modulate the baroreceptor heart rate reflex in conscious rats. Brain Res, 494(2), 232-240.

Liang, KC. And Chiang, TC. (1994) Locus coeruleus infusion of clonidine impaired retention and attenuated memory enhancing effects of epinephrine. Society for Neuroscience Abstracts, 20, 153.

Longhurst, J.C. (2003). Neural Regulation of the Cardiovascular System. In Fundamental Neuroscience. Edited by Squire, Bloom, Spitzer, Zigmond, Roberts, McConnell. Academic Press, 935-965.

Malkova, L., Gaffan, D., & Murray, E. A. (1997). Excitotoxic lesions of the amygdala fail to produce impairment in visual learning for auditory secondary reinforcement but interfere with reinforcer devaluation effects in rhesus monkeys. J Neurosci, 17(15), 6011-6020.

McDonald, R. J., & White, N. M. (1995). Information acquired by the hippocampus interferes with acquisition of the amygdala-based conditioned-cue preference in the rat. Hippocampus, 5(3), 189-197. McGaugh, J. L., Cahill, L., & Roozendaal, B. (1996). Involvement of the amygdala in memory storage: interaction with other brain systems. Proc Natl Acad Sci U S A, 93(24), 13508-13514.

McGaugh, J.L., Introini-Collison ,I.B., Cahill, L., Kim, M., Liang, K.C.(1992). Involvement of the amygdala in neuromodulatory influences on memory storage.

In The Amygdala. Edited by JP Aggleton. New York City: Wiley-Liss; (1992):431-452.

Miyashita, T., & Williams, C. L. (2002). Glutamatergic transmission in the nucleus of the solitary tract modulates memory through influences on amygdala noradrenergic systems. Behav Neurosci, 116(1), 13-21.

Nakamura K, Ono T, Fukuda M, Uwano T. Paraventricular neuron chemosensitivity and activity related to blood pressure control in emotional behavior. J Neurophysiol. 1992 Feb;67(2):255-64.

Parkinson, J. A., Cardinal, R. N., & Everitt, B. J. (2000). Limbic cortical-ventral striatal systems underlying appetitive conditioning. Prog Brain Res, 126, 263-285.

Petrovich GD, Holland PC, Gallagher M. Amygdalar and prefrontal pathways to the lateral hypothalamus are activated by a learned cue that stimulates eating. J Neurosci. 2005 Sep 7;25(36):8295-302.

Powell, D. A., Chachich, M., Murphy, V., McLaughlin, J., Tebbutt, D., & Buchanan, S. L. (1997). Amygdala-prefrontal interactions and conditioned bradycardia in the rabbit. Behav Neurosci, 111(5), 1056-1074.

Price JL, Fokje TR, Amaral, DG. (1987) The limbic region. II: The amygdaloid complex. In Handbook of Chemical Neuroanatomy, edited by Bjorklund, Hokfelt, Swanson. Elsevier Science Publishers. 279-388.

Price, J.L. (2003). Comparative aspects of amygdala connectivity. Ann N Y Acad Sci. Apr;985:50-8.

Reis DJ, Ledoux JE. (1987) Some central neural mechanisms governing resting and behaviorally coupled control of blood pressure. Circulation.Jul;76(1 Pt 2):I2-9.

Ricardo, J. A., & Koh, E. T. (1978). Anatomical evidence of direct projections from the nucleus of the solitary tract to the hypothalamus, amygdala, and other forebrain structures in the rat. Brain Res, 153(1), 1-26.

Roozendaal, B. (2000). 1999 Curt P. Richter award. Glucocorticoids and the regulation of memory consolidation. Psychoneuroendocrinology, 25(3), 213-238.

Roozendaal, B., Williams, C. L., & McGaugh, J. L. (1999). Glucocorticoid receptor activation in the rat nucleus of the solitary tract facilitates memory consolidation: involvement of the basolateral amygdala. Eur J Neurosci, 11(4), 1317-1323.

Rybak IA, Rogers RF, Schwaber JS. (2002) Modeling the baroreceptor vagal reflex. Neural Computation Program DuPont de Nemours & Co., Inc., Wilmington, DE http://www.rybak-et-al.net/baro.html

Saha S, Batten TF, Henderson Z. A GABAergic projection from the central nucleus of the amygdala to the nucleus of the solitary tract: a combined anterograde tracing and electron microscopic immunohistochemical study. Neuroscience. 2000;99(4):613-26.

Saha S. Role of the central nucleus of the amygdala in the control of blood pressure: descending pathways to medullary cardiovascular nuclei.

Sahgal A. A critique of the vasopressin-memory hypothesis. Psychopharmacology (Berl). 1984;83(3):215-28.

Sajdyk, T. J., & Shekhar, A. (1997). Excitatory amino acid receptor antagonists block the cardiovascular and anxiety responses elicited by gamma-aminobutyric acidA receptor blockade in the basolateral amygdala of rats. J Pharmacol Exp Ther, 283(2), 969-977.

Sato MA, Schoorlemmer GH, Menani JV, Lopes OU, Colombari E. Recovery of high blood pressure after chronic lesions of the commissural NTS in SHR. Hypertension. 2003 Oct;42(4):713-8. Epub 2003 Aug 4.

Shekhar, A., Sajdyk, T. S., Keim, S. R., Yoder, K. K., & Sanders, S. K. (1999). Role of the basolateral amygdala in panic disorder. Ann N Y Acad Sci, 877, 747-750.

Soltis, R. P., Cook, J. C., Gregg, A. E., & Sanders, B. J. (1997). Interaction of GABA and excitatory amino acids in the basolateral amygdala: role in cardiovascular regulation. J Neurosci, 17(23), 9367-9374.

Swanson, L. W., & Petrovich, G. D. (1998). What is the amygdala? Trends Neurosci, 21(8), 323-331.

Taylor, B. K., Holloway, D., & Printz, M. P. (1994). A unique central cholinergic deficit in the spontaneously hypertensive rat: physostigmine reveals a bradycardia associated with sensory stimulation. J Pharmacol Exp Ther, 268(3), 1081-1090.

Valentino, Rita J.; Aston-Jones, Gary S. (1998). Physiological and anatomical determinants of locus coeruleus discharge: Behavioral and clinical implications. Bloom, F. E. Kupfer, D. J., In: Psychopharmacology: The fourth generation of progress. Raven Press ; 1185 Avenue of the Americas, New York, New York 10036-2806, USA, 1995. 373-385.

Van Bockstaele, E. J., Colago, E. E., & Valentino, R. J. (1998). Amygdaloid corticotropin-releasing factor targets locus coeruleus dendrites: substrate for the co-ordination of emotional and cognitive limbs of the stress response. J Neuroendocrinol, 10(10), 743-757.

Van Wimersma Greidanus TB, Jolles J, De Wied D. (1985) Hypothalamic neuropeptides and memory. Acta Neurochir (Wien).;75(1-4):99-105.

Whalen, P. J., Rauch, S. L., Etcoff, N. L., McInerney, S. C., Lee, M. B., & Jenike, M. A. (1998). Masked presentations of emotional facial expressions modulate amygdala activity without explicit knowledge. J Neurosci, 18(1), 411-418.

White, N. M., & McDonald, R. J. (1993). Acquisition of a spatial conditioned place preference is impaired by amygdala lesions and improved by fornix lesions. Behav Brain Res, 55(2), 269-281.

White, N. M., Packard, M. G., & Hiroi, N. (1991). Place conditioning with dopamine D1 and D2 agonists injected peripherally or into nucleus accumbens. Psychopharmacology (Berl), 103(2), 271-276.

Young, B. J., & Leaton, R. N. (1996). Amygdala central nucleus lesions attenuate acoustic startle stimulus-evoked heart rate changes in rats. Behav Neurosci, 110(2), 228-237.

VI

Discussion and Conclusions

1. Discussion: Associative Learning

This body of work represents a step towards addressing an overarching hypothesis that the encounter of affectively significant learning situations elicits fast adaptive responses regulated by subcortical associative learning loci. The work presented herein suggests that adaptive responses to affectively significant situations arise as a result of activity in a broad range of neural structures, all partially recruited by information processing in the basolateral amygdala. Basolateral amygdala function seems to be integral in the regulation of a continuous state of adaptation by reacting quickly and appropriately to affective stimuli.

The particular influence that the BLA exerts in regulation of associative learning and affective arousal was explored and discussed in detail in the previous chapters of this thesis. Here the different contributions of the BLA to cognitive and behavioral neural processing are integrated in an attempt to present a coherent theory of BLA function in affective associative learning and arousal.

One of the experiments discussed in previous chapters employed a behavioral learning task that included gradations of both appetitive and aversive stimuli, spanning a sector of the valence continuum. The results of this associative learning experiment suggest that the basolateral amygdala is a fast associative learning center that participates in the process of coding the relative values of both positive and aversive affective stimuli. Evidence that the BLA is important for the recognition and association of stimuli with a range of both positive and aversive values supports a hypothesis by Rolls (Rolls, 1999).

This hypothesis emphasizes the necessity for organisms to recognize a "reward currency" of stimuli in the environment (Rolls, 1999). The further importance of recognizing gradations in both rewards and punishments on a valence continuum is emphasized here. The integrity of the basolateral amydala is absolutely essential to choosing between items holding values that are close to each other on this spectrum.

Very early in associative learning, BLA neurons demonstrate selectivity for particular visual objects holding different values (Quinn et al., in preparation). In the present study, the earliest behavioral change to emerge across learning is appropriate instrumental approach and avoidance behavior. Accurate choice behavior is evident later in learning, at a time point that far exceeds the intial selectivity of BLA neurons. This later contribution of the BLA to choice behavior is consistent with the theory that the interconnectivity between the OFC and the BLA, as well as the OFC by itself, are particularly important for goal directed judgements and behaviors (Baxter et al., 2000; Baxter and Murray, 2002; Izquierdo and Murray, 2004). There exists evidence that the BLA is important for tuning the selectivity of neurons in the OFC (Schoenbaum et al., 2003), in addition to evidence that the OFC and amygdala work together in determining the current value of a stimulus (Baxter et al., 2000). The early contribution of the BLA to appropriate instrumental responding is likely to be consistent with the existence of a link between the relative sensory value and the motivational value of affective stimuli, supported by several authors in their theories about the role for the amygdala in learning (Winston et al., 2005; Balleine et al., 2005).

Both the instrumental learning results and the choice behavior results clearly demonstrate that the basolateral amygdala is necessary for distinguishing between

negatively associated objects early in training. During the first several trials of instrumental learning, vehicle rats begin to preferentially avoid the object with the worst outcome, whereas BLA rats avoid it to a lesser degree. This is completely in line with extensive research on the role of the BLA in aversive associative learning, including fear conditioning and passive avoidance learning (Armony et al., 1995; Davis, 1997; LeDoux, 1993; Davis, 1992; McGaugh et al., 1992). Lateral amygdala neurons demonstrate almost immediate selectivity to conditioned fearful stimuli (Quirk et al., 1997), further supporting a role for the amygdala in facilitating rapid acquisition of stimulus relevance.

Our results suggest that appropriate behavioral outcomes after extended training time are only partially dependent on the basolateral amygdala. The extent of the BLA influence on late learning seems to be determined by the relative distance between the affective value of the associated stimuli on the valence scale. This limitation is apparent in the fact that the BLA lesioned animals partially master learning the most distant object – valence associations by the last day of training. This is demonstrated by partial improvement in performance on the choice behavior and by the late emergence of appropriate instrumental responses to objects in the BLA lesioned rats. This result supports the findings from the literature that alternate brain structures important for decision making and goal directed behavior, such as the orbitofrontal cortex, that engage more slowly than the amygdala, actively participate in the affective associative learning process (Baxter et al., 2000; Baxter and Murray, 2002; Schoenbaum et al., 1999). In the absence of the basolateral amygdala, other ventral striatal structures, including the nucleus accumbens and the ventral tegmental area in addition to the striatopallidal circuitry are sufficient for encoding a portion of that which they would ordinarily encode

in concert with the amygdala (White, 1989; Everitt et al., 1989; Everitt et al., 1991; Everitt and Robbins, 1992).

The afore mentioned emergence of instrumental learning of object – valence associations precedes the emergence of appropriate choice behaviors in vehicle rats. One of the factors driving the distribution of instrumental responses to objects paired with different values is the degree of arousal associated with rewarding and aversive reinforcements. The latency of approach data for very early training shows that animals run faster to the more "arousing" objects, associated with more extreme appetitive and aversive food values. Putatively, the animals' increased arousal levels improve their memory for the objects, which is highly consistent with the extensive research from the McGaugh laboratory showing that the BLA is imperative in improving memory for highly arousing events (McGaugh et al., 1996, Roozendaal, 2000; Roozendaal et al., 1999; Ferry et al., 1999c, Miyashita and Williams, 2002; Curtis et al., 2002).

2. Discussion: Physiological State and Learning

While particular stimuli can elicit rapid changes in arousal, behavioral context can elicit more stable baseline shifts in arousal levels. There exists a relationship between physiological arousal and contingent contexts in learning, as demonstrated using a BLA dependent appetitive paradigm. Overall, the animals' arousal level seemed to be high during exposure to a non-contingent appetitive environment, and then decreased following a transfer to a contingent appetitive environment. Thus, it was determined that learning of the contingencies in the appetitive context had the most striking attenuating effect on blood pressure. The integrity of the basolateral amygdala was essential for

learning the reinforcement contingencies in this environment. However, in the absence of the basolateral amygdala, the arousal level, as measured by blood pressure, was only marginally decreased during learning of contingencies, indicating a partial contribution of the BLA to this state modulation.

An appropriately decreased level of arousal when shifting to contingent environments may set the stage for place preference formation. Whereas the integrity of the amygdala is essential for place preference formation, there are many structures besides the BLA, such as the hippocampal formation and the striatopallidal circuitry that enable place preference learning (White, 1989; White and McDonald, 1993; McDonald and White, 1995; Everitt et al., 1991). The interplay of these structures has not been elaborated in place preference formation, but clues from the stress literature may shed light on the circuit. As evident from the literature on the interaction between stress and behavior, acute stress, supplemented by high arousal levels, impairs hippocampal function and dysregulates the function of the basal ganglia circuitry (Gould et al., 1997; McEwen, 2000; Roberts et al., 2000). Conversely, lower arousal levels could correlate with efficient hippocampal and striatal functioning during place preference conditioning. Thus, the appropriately decreased levels of arousal that were observed in a contingent appetitive environment may have served to facilitate place preference formation by preserving regulation of the underlying circuitry.

3. Discussion: The amygdala, allostasis, and adaptation

The interaction between level of physiological arousal of an organism and learning may go awry during chronically altered levels of physiological arousal, such as

those seen in hypertension. When an organism has a genetic tendency, or a predisposition, for hypertension, associative learning processing may potentially be altered. This is consistent with both behavioral and neurochemical alterations that have been documented in the literature on aversive conditioning in hypertensive and normotensive rats (LeDoux et al., 1986; Conti and Printz, 2003; Taylor et al., 1994).

Global state changes, including the emergence of hypertension, have been reliably correlated with allostatic load and allostatic processing (Sterling and Eyer, 1988; McEwen, 2000; Koob and LeMoal, 2001). The chapter on the neural regulation of allostatic processing discusses at length how allostatic dysregulation can give rise to changes in the brain on structural, neurochemical, and behavioral levels. The basic purpose of allostasis is to help the organism adapt to adverse circumstances, and to accommodate to demands from both the external and the internal environments (McEwen, 2000; Koob and LeMoal, 2001). The circuitry implicated in the regulation of allostatic processing has the amygdala and its associated structures at its heart (Koob and LeMoal, 2001). There are several logical implications that arise from this theoretical construct. One of the implications, discussed in the chapter on allostatic processing, is that the functions subserved by the amygdala are upregulated during elevated demands on the system in the form of acute stress or arousal, in the face of downregulaton of other structures in the circuit (McGaugh et al., 1996, Roozendaal, 2000; Roozendaal et al., 1999; Ferry et al., 1999; Quirarte et al., 1997; Miyashita and Williams, 2002). Another broad implication is that one of the purposes of basolateral amygdala function, including its role as the fast associator of affective stimuli, is to help the organism adapt to the changing demands of the environment.

4. Conclusion

Rapid learning, in which the amygdala plays a role, is essential to adaptation, even in relation to large-scale social issues that are presumably evolutionary in origin. Some such broad adaptive effects can be observed in everyday life, for example, the "race effect" – the prevalent inability of people to visually differentiate faces of alternate racial origins – where amygdalar activation has been implicated (Phelps et al., 2000; Hart et al., 2000). Faces are deemed highly relevant social cues and appropriate social responding is dependent on rapid evaluation of faces. When the time scale is protracted, face detection is altered, such that the "race effect" is minimized or abolished by extensive training that leads to expertise (Tanaka et al., 2004).

The close and functionally enigmatic relationship of the BLA to the visual cortex, is likely to provide a venue through which relevance can directly bias visual perception. In the rats newly constructed world, the fast and uniform approach latencies of the BLA lesioned rats to affectively associated objects early in training could be consistent with this basic lack of the visual skills for telling apart affectively salient stimuli. This assertion underscores the importance of the communication between the amygdala and the visual system in rapidly coding the sensory properties of potentially relevant stimuli.

As discussed extensively throughout this thesis, the basolateral complex of the amygdala does not function in isolation, but does so through its interconnectivity with many different brain systems. The neuroanatomical framework defined in this thesis may illustrate how neural circuitry for associative learning, neural circuitry for motivational salience, and neural circuitry for regulation of cardiovascular activity could directly

interact. This framework has a large degree of overlap with, and was partially based on the connectivity between, structures implicated in allostatic regulation (McEwen, 2000; Koob and LeMoal, 2001).

This anatomical model provides a rationale for how the amygdala, including the basolateral amygdala, could participate in the emergence of global state changes that help the organism adapt to its environment on behavioral, cognitive, and physiological levels. The basolateral amygdala assumes a particularly important role in this model as it is very fast at forming affectively significant associations and potentially drives persistent changes in associated circuitry.

In this thesis we have tested and integrated a number of hypotheses on associative learning, affect, arousal and organismal function in the environment. The findings presented here are in support of a theoretical construct emphasizing a pivotal role of the extended amygdala and related subcortical structures in associative learning and adaptation to the environment. Being able to adjust and adapt to novel circumstances requires the whole brain, but the subcortical associative learning loci enable the fluidity and speed of adaptation.

5. References

Armony, J. L., Servan-Schreiber, D., Cohen, J. D., & LeDoux, J. E. (1995). An anatomically constrained neural network model of fear conditioning. Behav Neurosci, 109(2), 246-257.

Balleine BW. Neural bases of food-seeking: Affect, arousal and reward in corticostriatolimbic circuits. Physiol Behav. 2005 Dec 15;86(5):717-30. Epub 2005 Oct 27

Baxter, M. G., & Murray, E. A. (2002). The amygdala and reward. Nat Rev Neurosci, 3(7), 563-573.

Baxter, M. G., Parker, A., Lindner, C. C., Izquierdo, A. D., & Murray, E. A. (2000). Control of response selection by reinforcer value requires interaction of amygdala and orbital prefrontal cortex. J Neurosci, 20(11), 4311-4319.

Conti LH, Printz MP. Rat strain-dependent effects of repeated stress on the acoustic startle response. Behav Brain Res. 2003 Sep 15;144(1-2):11-8

Curtis AL, Bello NT, Connolly KR, Valentino RJ. Corticotropin-releasing factor neurones of the central nucleus of the amygdala mediate locus coeruleus activation by cardiovascular stress. J Neuroendocrinol. 2002 Aug;14(8):667-82.

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. J Neuropsychiatry Clin Neurosci, 9(3), 382-402.

Everitt, B. J., Cador, M., & Robbins, T. W. (1989). Interactions between the amygdala and ventral striatum in stimulus-reward associations: studies using a second-order schedule of sexual reinforcement. Neuroscience, 30(1), 63-75.

Everitt, B.J., Robbins, T,W. (1992). Amygdala-ventral striatal interactions and

Gould, E; B.S. McEwen, P. Tanapat, L.A.M. Galea and E. Fuchs, Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. J. Neurosci. 17 (1997), pp. 2492-2498.

Hart AJ, Whalen PJ, Shin LM, McInerney SC, Fischer H, Rauch SL. Differential response in the human amygdala to racial outgroup vs ingroup face stimuli. Neuroreport. 2000 Aug 3;11(11):2351-5

Izquierdo A, Murray EA. Combined unilateral lesions of the amygdala and orbital prefrontal cortex impair affective processing in rhesus monkeys. J Neurophysiol. 2004 May;91(5):2023-39. Epub 2004 Jan 7

Koob GF. Neuroadaptive mechanisms of addiction: studies on the extended amygdala. Eur Neuropsychopharmacol. 2003 Dec;13(6):442-52.

Koob, G.F. and Le Moal, M., 1997. Drug abuse: Hedonic homeostatic dysregulation. Science 278, pp. 52-58.

LeDoux JE, Sakaguchi A, Reis DJ. Strain differences in fear between spontaneously hypertensive and normotensive rats. Brain Res. 1983 Oct 24;277(1):137-43.

LeDoux, J. E. (1993). Emotional memory systems in the brain. Behav Brain Res, 58(1-2), 69-79.

LeDoux, J. E., Iwata, J., Pearl, D., & Reis, D. J. (1986). Disruption of auditory but not visual learning by destruction of intrinsic neurons in the rat medial geniculate body. Brain Res, 371(2), 395-399.

McDonald, R. J., & White, N. M. (1995). Information acquired by the hippocampus interferes with acquisition of the amygdala-based conditioned-cue preference in the rat. Hippocampus, 5(3), 189-197.

McEwen, BS. Allostasis and allostatic load: implications for neuropsychopharmacology Neuropsychopharmacology, Feb 2000(a), 22(2):108-24.

McGaugh, J. L., Cahill, L., & Roozendaal, B. (1996). Involvement of the amygdala in memory storage: interaction with other brain systems. Proc Natl Acad Sci U S A, 93(24), 13508-13514.

McGaugh, J.L., Ferry B, Vazdarjanova, A, Roozendaal, B. (2000). Amygdala: role in modulation of memory storage. In The Amygdala. Edited by JP Aggleton. New York City: Oxford University Press, 391-412.

McGaugh, J.L., Introini-Collison ,I.B., Cahill, L., Kim, M., Liang, K.C.(1992). Involvement of the amygdala in neuromodulatory influences on memory storage. In The Amygdala. Edited by JP Aggleton. New York City: Wiley-Liss; (1992):431-452.

Miyashita, T., & Williams, C. L. (2002). Glutamatergic transmission in the nucleus of the solitary tract modulates memory through influences on amygdala noradrenergic systems. Behav Neurosci, 116(1), 13-21.

Phelps EA, O'Connor KJ, Cunningham WA, Funayama ES, Gatenby JC, Gore JC, Banaji MR. Performance on indirect measures of race evaluation predicts amygdala activation. J Cogn Neurosci. 2000 Sep;12(5):729-38

Quinn L.K., Merzlyak I.Y., Minces V., Chiba A.A. Neural Activity in the Rat Basolateral Amygdala Reflects the Acquired Motivational Significance of Visual Objects (in preparation).

Quirarte, G.L., Roozendaal, B. and McGaugh, J.L., 1997. Glucocorticoid enhancement of memory storage involves noradrenergic activation in the basolateral amygdala. Proceedings of the National Academy of Sciences, USA. 1997 Dec 9, 94(25):14048-53.

Quirk GJ, Armony JL, LeDoux JE. Fear conditioning enhances different temporal components of tone-evoked spike trains in auditory cortex and lateral amygdala. Neuron. 1997 Sep;19(3):613-24.

Ray, R. D. (1977). Physiological-behavioral coupling research in the Soviet science of higher nervous activity: a visitation report. Pavlov J Biol Sci, 12(1), 41-50.
reward-related processes. In The Amygdala. Edited by JP Aggleton. New York City: Wiley-Liss; 401-430.

Roberts, AJ; Heyser, CJ; Cole, M; Griffin, P; Koob, GF. Excessive ethanol drinking following a history of dependence: animal model of allostasis. Neuropsychopharmacology, 2000 Jun, 22(6):581-94.

Rolls, E.T., (1999). The Brain and Emotion. Oxford University Press, Oxford, England.

Roozendaal, B. (2000). 1999 Curt P. Richter award. Glucocorticoids and the regulation of memory consolidation. Psychoneuroendocrinology, 25(3), 213-238.

Roozendaal, B., Williams, C. L., & McGaugh, J. L. (1999). Glucocorticoid receptor activation in the rat nucleus of the solitary tract facilitates memory consolidation: involvement of the basolateral amygdala. Eur J Neurosci, 11(4), 1317-1323.

Schoenbaum G, Setlow B, Saddoris MP, Gallagher M. Encoding predicted outcome and acquired value in orbitofrontal cortex during cue sampling depends upon input from basolateral amygdala. Neuron. 2003 Aug 28;39(5):855-67.

Schoenbaum, G., Chiba, A. A., & Gallagher, M. (1999). Neural encoding in orbitofrontal cortex and basolateral amygdala during olfactory discrimination learning. J Neurosci, 19(5), 1876-1884.

Sterling, Peter; Eyer, Joseph Allostasis: A new paradigm to explain arousal pathology. In: Shirley Fisher, Ed; James Reason, Ed; et al. Handbook of life stress, cognition

and health. John Wiley & Sons: Chichester, England UK, 1988. p. 629-649 of xxxiii, 750pp.

Tanaka JW, Kiefer M, Bukach CM. A holistic account of the own-race effect in face recognition: evidence from a cross-cultural study. Cognition. 2004 Aug;93(1):B1-9

Taylor, B. K., Holloway, D., & Printz, M. P. (1994). A unique central cholinergic deficit in the spontaneously hypertensive rat: physostigmine reveals a bradycardia associated with sensory stimulation. J Pharmacol Exp Ther, 268(3), 1081-1090.

Vuilleumier P, Richardson MP, Armony JL, Driver J, Dolan RJ, Distant influences of amygdala lesion on visual cortical activation during emotional face processing, Nat Neurosci. 2004 Nov;7(11):1271-8. Epub 2004 Oct 24.

White NM, McDonald RJ. Acquisition of a spatial conditioned place preference is impaired by amygdala lesions and improved by fornix lesions. Behav Brain Res. 1993 Jun 30;55(2):269-81

White, N. M. (1989). Reward or reinforcement: what's the difference? Neurosci Biobehav Rev, 13(2-3), 181-186.

Winston JS, Gottfried JA, Kilner JM, Dolan RJ. Integrated neural representations of odor intensity and affective valence in human amygdala. J Neurosci. 2005 Sep 28;25(39):8903-7.