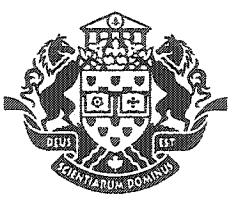
NOTE TO USERS

Page(s) not included in the original manuscript and are unavailable from the author or university. The manuscript was scanned as received.

102

This reproduction is the best copy available.

UMI®



Université d'Ottawa · University of Ottawa

Université d'Ottawa · University of Ottawa

FACULTÉ DES ÉTUDES SUPÉRIEURES ET POSTDOCTORALES

FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

MIGUELEZ, Maïa

AUTEUR DE LA THÈSE - AUTHOR OF THESIS

Ph.D (Psychology)

GRADE - DEGREE

School of Psychology

FACULTÉ, ÉCOLE, DÉPARTEMENT - FACULTY, SCHOOL, DEPARTMENT

TITRE DE LA THÈSE - TITLE OF THE THESIS

The Functional Significance of Cortical and Adjacent Amygdaloid Sub-Nuclei in the Modulation of Brain Stimulation Reward Derived from the Medial Forebrain Bundle

Kate Bielajew

DIRECTEUR DE LA THÈSE - THESIS SUPERVISOR

EXAMINATEURS DE LA THÈSE - THESIS EXAMINERS

G. Fouriezos

C. Plowright

C. Messier

M. Waraczunsky

J.-M. De Koninck, Ph.D.

LE DOYEN DE LA FACULTÉ DES ÉTUDES SUPÉRIEURES ET POSTDOCTORALES

SIGNATURE

DEAN OF THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

The functional significance of cortical and adjacent amygdaloid sub-nuclei in the modulation of brain stimulation reward derived from the medial forebrain bundle

Maïa Miguelez

Thesis submitted to the
Faculty of Graduate and Postdoctoral Studies
in partial fulfillment of the requirements
for the Ph.D degree in Psychology

School of Psychology
Faculty of Social Sciences
University of Ottawa

Your file Votre référence
ISBN: 0-494-01738-4
Our file Notre référence
ISBN: 0-494-01738-4

DEDICATION

This thesis is dedicated to three people: my husband, my daughter, and my supervisor.

Nico: I will never be able to fully convey my gratitude and love towards you. Thank you for always supporting me unconditionally, for being my inspiration and my best friend and for making my life such a happy, magical, and fulfilling adventure.

Anna: You were born on the same year of this dissertation! Sweet baby, this thesis, which is the second greatest accomplishment in my life, is dedicated to you, my most important one.

Kate: I am endlessly grateful to you for passing on to me such an extensive wealth of knowledge with patience, kindness, and humour. I consider myself fortunate to have been supervised by a person who is both a brilliant scientist and an exceptional person. Thank you from the bottom of my heart, dearest Kate, for your scholarly guidance, unwavering support, kind concern, and friendship throughout the years. Thank you for always looking out for me, for protecting my interests and for caring so much about my well-being. You have been a remarkable mentor and, just as in the case of Anne, my guardian angel.

ACKNOWLEDGEMENTS

There are many people that I would like to acknowledge.

My beloved parents and brother have always given me their unconditional love and encouragement. Your pride and support have sustained me throughout this and all of my life's experiences. I especially owe a debt of gratitude to my father, for his advice and support these past years. It is thanks to him that on many occasions I have been able to vent the day's frustrations while having healthy and balanced meals upon returning exhausted from the lab!

My lab partners have encouraged and helped me on many occasions. Thank you Magali, Angela, Stephanie and Mandy, for your companionship and laughs. Mandy, I am particularly grateful to you for patiently and graciously helping me out with my last manuscripts. I especially owe a debt of gratitude to my dear friend and senior lab partner Anne - not only have you been a rock for me personally through good and bad times, but you have also taught me everything you know with exceptional dedication, patience, and kindness. I will never forget everything you have done for me.

The School of Psychology personnel have been wonderful throughout all my years in the graduate program. A special thanks to Mireille, Jeanne d`Arc, Louise, France, and more recently Julie for always being so nice and caring. Sylvie, your help and friendly support have been tremendously appreciated. Thank you for always knowing what to do and for helping out.

Pierre, thank you for your help with the figures, for your good humour and for always accommodating my hectic schedule. You are wonderful!

Last but not least, I want to thank my committee members for taking the time to support my work. I am very grateful for your valuable feedback and kindness.

ABSTRACT

The primary objective of my thesis is to explore the involvement of the amygdala, a neural structure underlying emotion, in the circuit underlying motivated behaviour as modelled by brain stimulation reward (BSR). In the first experiment, the functional links between the lateral hypothalamus (LH) and the amygdala were assessed using the metabolic marker, cytochrome oxidase. Following lesions of LH sites that supported BSR, we obtained a significant reduction of oxidative metabolism in the cortical sub-nuclei of the amygdala, suggesting their involvement in modulating the reward signals elicited from the LH. In order to test this functional relationship further, we extended our exploration of this link to another reward site, the ventral tegmental area (VTA), on both hemispheres of the brain. Following lesions to the cortical nuclei of the amygdala, some animals exhibited significant and sustained decreases in the rewarding value of LH as well as of VTA stimulation. In addition, modifications in the rewarding value of the stimulation from the MFB site contralateral to the lesion resembled ipsilateral ones, suggesting the existence of interhemispheric links in amygdaloid alteration of MFB reward signals. The nature of interhemispheric connectivity as well as a more detailed investigation of the amygdaloid sites involved in the modulation of MFB reward were explored in the final study of the thesis. Thresholds for BSR obtained from a continuum of MFB sites were tracked following serial bilateral lesions to the amygdala. One group of animals showed marked increases in thresholds that further augmented or were maintained after the second lesion. Damage to the most anterior sites encompassing cortical amygdaloid nuclei were confirmed as being more efficient than other lesions in producing substantial threshold changes. Equally exceptional was the finding that contralateral lesions were more effective than ipsilateral ones in

affecting thresholds, confirming our earlier finding of interhemispheric connectivity. This interpretation was corroborated in a metabolic sub-study using cytochrome oxidase. Reaction product density within lesion sites was significantly and negatively correlated with substantial decreases in reward from the MFB electrode contralateral to them. Taken together, these data indicate that the anterior portions of the amygdaloid cortical nuclei modulate MFB reward signals. Furthermore, a significant interhemispheric communication appears to occur between these sub-nuclei in the context of reward.

TABLE OF CONTENTS

LIST OF ACRONYMS

ACh	Acetylcholine
AChE	Acetylcholinesterase
BNST	Bed nucleus of the stria terminalis
BSR	Brain stimulation reward
2-DG	2-Deoxyglucose
LH	Lateral hypothalamus
MFB	Medial forebrain bundle
VTA	Ventral tegmental area

INTRODUCTION

1. BRAIN STIMULATION REWARD

1.1 THEORETICAL CONSIDERATIONS ABOUT REWARD AND MOTIVATION

"Quelle chose étrange, mes amis, paraît être ce qu'on appelle le plaisir! Et quel singulier rapport il a naturellement avec ce qui passe pour être son contraire, la douleur! Ils refusent de se rencontrer ensemble chez l'homme; mais qu'on poursuive l'un et qu'on l'attrape, on est presque toujours contraint d'attraper l'autre aussi, comme si, en dépit de leur dualité, ils étaient attachés à une seule tête." Phédon, 370 BC, in Platon, 1965, p. 107.

Interpreting such concepts as "emotion", "affect", "hedonism", and "reward" is an ongoing journey that is almost as old as humanity itself. In the course of this incessant quest for understanding, philosophers and scientists have devised hundreds of ideas and theories to demystify such notions. A recurrent element in this line of questioning is that of the tight link between these constructs and that of motivation, operationally conceived as motivated behaviour. A great number of theoretical explanations converge on the idea that behaviour is vastly determined by its emotional consequences, usually defined along a continuum that goes from aversive (unpleasant, painful, distressing) to appetitive (pleasant, satiation-producing, rewarding). In other words, and according to various theories of reinforcement, there exists an important similarity between positive and negative reinforcement processes in that they are both strong modulators of adaptive activity (Kiyatkin, 1989).

Although the existence of "positive" and "negative" emotions is undisputable, modern

public and scholarly conceptualization appears to generally consider only the negative emotions as worthy of scientific inquiry. In the context of this "philosophy of lack" (Fonberg, 1972), researchers more readily embrace stress than reward, or pain than pleasure, despite the accumulating evidence showing that both positive and negative emotions are connected with biological needs (Fonberg, 1972). Moreover, at a physiological level, strikingly similar processes have been observed to occur in response to aversive as well as to appetitive events (Bielajew and Shizgal, 1980; Merali et al., 1998). Following these considerations, it is not unreasonable to speculate that all motivated behaviours stem from a common substrate that is governed by the necessity of providing physiological equilibrium. Embracing this assumption allows us not only to define goal-directed actions, but also to conceptualize a diverse range of behaviours into one theoretical model (Stellar and Stellar, 1985).

The governing postulate underlying the research, theoretical concepts, and ideas presented in this dissertation stems from the logic that motivation is a definable attribute of behaviour and that behaviour is the result of activity in the brain (Yeomans, 1990). The concept of motivation can thus be operationally conceptualized as the arousal of certain networks of the nervous system, which elicit specific emotional as well as physiological states leading to the execution of instrumental acts (adapted from Fonberg, 1972). Behaviours that are motivated by reward are believed to be neurologically related so that their organization follows the same physiological laws (Stellar and Stellar, 1985). These laws can in turn be investigated by means of physiological methods (Fonberg, 1972).

1.2 BRAIN STIMULATION REWARD AS A MODEL FOR MOTIVATED BEHAVIOUR

"*So pleasure, we are told, like happiness or interest, can never be fixed directly by the naked eye - let alone pursued as an end, or conceptualized - but only experienced laterally, or after the fact, as something like the by-product of something else.*" Jameson, F. (1983) p. 1.

A solution to the problem of finding an adequate paradigm to study the biological bases of reward was provided by Olds and Milner in 1954. These researchers showed that electrical stimulation of parts of the rat's limbic system appeared to be so pleasurable that the animals would self-administer it at extraordinarily high rates, a behaviour reminiscent of an addict's drug consumption. In fact, because animals also work to obtain opiate and stimulant drugs in several of the deep brain sites, it is believed that BSR may be related to drug reward (Olds and Fobes, 1981). One major difference with BSR, however, is that extinction of the operant behaviour is almost immediate following withdrawal of the stimulus. It is thus hypothesized that BSR produces simultaneously a state of "drive" (motivation) - established by a priming stimulus, and one of reward (reinforcement) - provided by the next stimulation (Valenstein et al., 1970).

Interestingly, BSR can be obtained from similar anatomical regions in such diverse species as the fish (Boyd and Gardner, 1962), snail (Balaban and Chase, 1989), bird (Goodman and Brown, 1966), cat (Justesen et al., 1963), dog (Stark and Boyd, 1961), and human (Bishop, Elder, and Heath, 1963), suggesting that reward plays a crucial role in orienting behaviour (Valenstein, 1973). In the rat, BSR can be elicited in sites comprising as much as one fifth of its total brain volume, with the frontal cortex as the most anterior loci and the cerebellum as the

most posterior one (Yeomans, 1990). However, this behaviour can be most easily drawn from the median forebrain bundle (MFB), a major fiber system that extends from the lateral hypothalamus (LH) to the ventral tegmental area (VTA) (Stellar and Stellar, 1985) (also see Appendix 1). In the rat, stimulation of the MFB elicits not only vigorous bar-pressing, but also other signs of positive affect such as sniffing, and exploration (Yeomans, 1990).

Over the last several years, the technique of BSR has evolved progressively to become highly sophisticated and quantitative, providing us with a unique model with which to study motivated behaviour. It combines the precision of mathematics by affording a tight control of the parameters used to electrically stimulate the brain on the one hand with the subjective experience of reward, defined by strict operational criterions (see below) on the other hand. These unique characteristics authorize the previously unconceivable notion of an objective and systematic investigation of the links between neural units and reward, with the ultimate goal of identifying the pathways and systems that underlie hedonic behaviours.

1.3. PARAMETRIC TECHNIQUES IN BRAIN STIMULATION REWARD

"How may we establish that activity in a neural system defined by electrophysiological and anatomical observation is the basis for the self-stimulation phenomenon? How may one support the claim that a particular [system] carries the neural signals mediating the rewarding or motivating effects of the stimulation? Our answer in brief is that neurophysiological and neuroanatomical techniques are, by themselves, incapable of solving the problem. One must establish by behavioral experiments what kinds of neurophysiological, neuroanatomical, and neuropharmacological attributes are to be looked for." Gallistel, Shizgal, and Yeomans (1981) p. 229.

Provided that it is correctly implemented, BSR is a technique that has the advantage of being minimally invasive, therefore ensuring that "normal" physiologic processes may be more readily approximated (Jankovic and Spector, 1986). The parametric conditions under which maximally effective BSR can be achieved have been the object of much research since the paradigm's discovery in 1954. For instance, cathodal stimulation of the brain has been shown to be more effective and safer than anodal current, which can lesion brain tissue via the secreting of metal ions from the electrode (Pecson, Roth, and Mark, 1969). Thus, fast-rising rectangular pulses of negative current are the preferred waveform administered for BSR.

Typically, rats learn to perform a certain task, such as lever pressing, in order to stimulate neurons at the tip of an electrode implanted in target sites of the brain (such as the MFB). Initially, the method of choice to quantify BSR was that of changes in rate of the

instrumental task used by the animals to self-administer the electrical stimulation. However, this index has since then been shown to be an unreliable measure of reward (Valenstein, 1964), since a decrease in the maximum rate could be related to factors unrelated to reward, such as motoric effects (Gallistel, Shizgal, and Yeomans, 1981). Thus, a reduction in the rate of lever-pressing, for instance, would not necessarily reflect a decrease in the rewarding value of the stimulation.

A more reliable index of reward has been developed, based on the threshold related to a fixed level of responding. A typical way to establish thresholds is to analyse rate-frequency curves, obtained by keeping one stimulation parameter constant and changing another one, in order to modify the value of the rewarding stimulation (see Figure 1). This value can be altered by reducing or increasing the frequency or number of pulses delivered, the intensity of the current, or the total duration of the train of pulses administered (Stellar and Stellar, 1985). Rate-frequency curves can be obtained, for instance, by holding the current constant and reducing the frequency of pulses from a value that produces the highest rate of bar-pressing to one that generates few or no responses. Thresholds are thereafter interpolated from the rate-frequency function and defined as the frequency corresponding to the half maximum rate of responding or a fixed criterion.

Figure 2 illustrates how reward and motoric factors can be differentiated using the method of threshold determination instead of that of rate (see Stellar and Stellar, 1985). An increase in the rewarding value of the stimulation is interpreted when the animal requires a fewer number of pulses, for instance, to produce a criterion performance of bar-pressing. Thus, in this case, threshold values shift to the left of the rate-frequency curve without affecting asymptotic level. Conversely, a higher threshold, illustrated by a rightward shift in the rate-frequency curve,

indicates a decrease in the rewarding value of the stimulation, again without necessarily changing the maximum rate of responding. Within limits, asymptotic values can increase or decrease as a result of performance variables. Thus, comparison of the frequency thresholds of the same pair of curves is a more reliable index of changes in rewarding value than the simple determination of maximum bar-presses (see Stellar and Stellar, 1985). All of these considerations are true if performance factors are held relatively constant (see Fouriezos, Bielajew, and Pagotto, 1990).

Figure 1. This figure depicts the relevant stimulation parameters in BSR. Typically, the train duration (500 ms) and the pulse duration (0.1 ms) are set at these constant values, whereas the frequency (or number of pulses) (N) in each train, and the current are modified. The period is the reciprocal of the frequency. Because frequency is defined as the number of pulses per second (Hz) and our train durations are usually less than this value, we may use instead the expression "number of pulses" or N rather than frequency to describe this parameter.

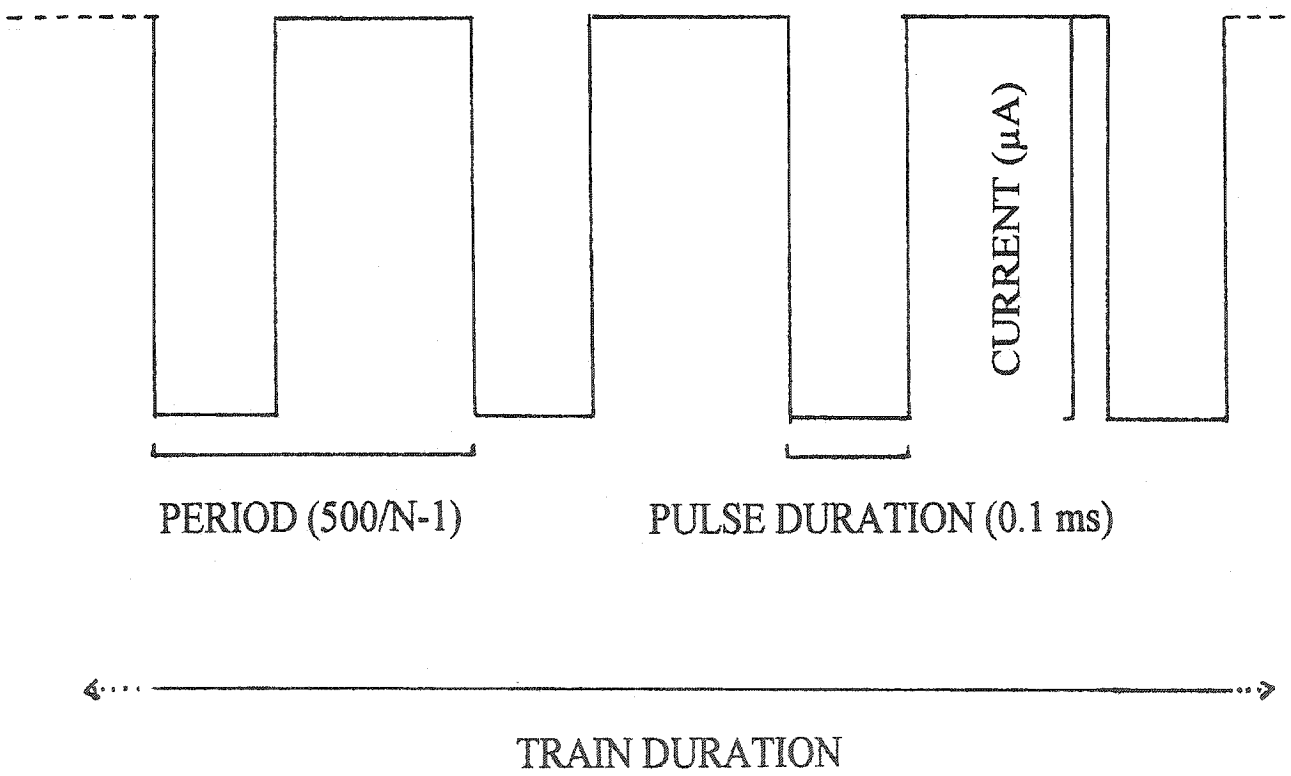
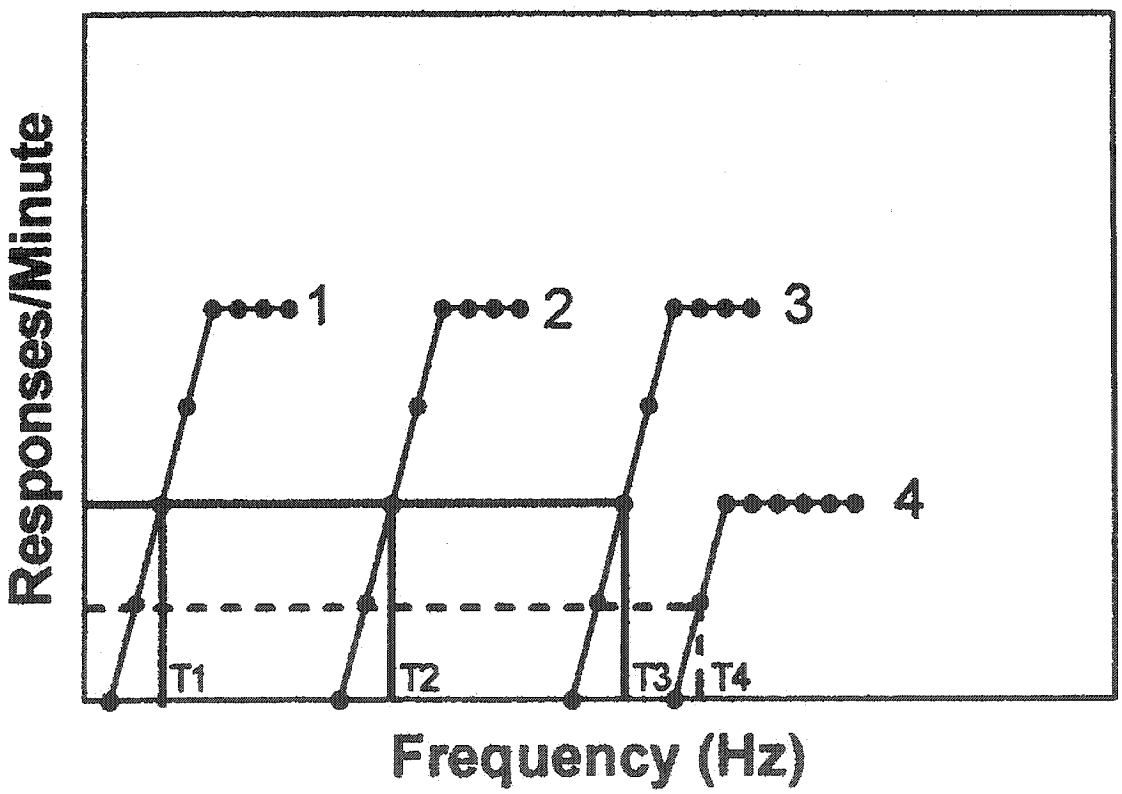


Figure 2. This figure illustrates how the value of the rewarding stimulation is dissociated from performance effects. In rate-frequency curves, the threshold values (T) correspond to the frequency associated with 50% of the maximum rate of response. Comparing curves 1 and 2, the latter illustrates a decrease in the rewarding value of the stimulation (shift to the right), with no modification of the maximum performance (asymptotic level). Conversely, curve 1 represents an increase in reward (shift to the left) in comparison with curve 2. When asymptotic levels change, such as is the case in curves 3 and 4, thresholds differ only very slightly, indicating a change in performance rather than one in reward. However, very significant changes in performance will alter the reward value (see Fouriezos, Bielajew, and Pagotto, 1990).



It is important to point out that the method of threshold determination is governed by two basic postulates. First, it is assumed that the population of neurons surrounding the tip of the electrode is homogenous, so that an increase in the stimulation current recruits a proportionally larger radius of similar neurons. Second, the number of action potentials of these neurons is comparable, so that an augmentation of frequency will increase the number of action potentials for all neurons in the vicinity of the stimulating electrode. It follows from these considerations that there is a strong relationship between stimulation parameters. For instance, an increase in the frequency of pulses delivered through the electrode augments the number of firings elicited in the stimulation area, amplifying the "value" of the rewarding stimulation. Another way to make the stimulation "more rewarding" is to make the neurons fire more rapidly by increasing the number of pulses (or decreasing the period) delivered through the electrode. Thus, it is assumed that current and frequency, or current and period, are inversely proportional, so that in order to maintain the same rewarding value, the first parameter must increase in magnitude while the second one must be decreased by the same degree (see Stellar and Stellar, 1985). Trade-off experiments of this type have been conducted and support the idea that varying stimulation parameters in an inversely proportional way elicit equivalent behavioural responses (Bielajew, Jurgens, and Fouriezos, 1987; Edmonds and Gallistel, 1974; Gallistel, 1974; Gallistel, Shizgal, and Yeomans, 1981; Malette and Miliaressis, 1990).

1.4. THE PURSUIT FOR THE SUBSTRATES OF BRAIN STIMULATION REWARD

"The circuit-like understanding of [brain stimulation reward] appears far off. Additionally, one must face the distinct possibility that such a circuit-like understanding may not, in reality, exist in any manageable form" Stellar and Stellar (1985) p. 121.

In order to understand how the brain elicits MFB reward, investigators have to attempt to find their way through a labyrinth of thousands of neurons that communicate via millions of synapses. Research questions that are this challenging are most efficiently tackled by the use of many different methods at various levels of analysis from the most basic (cellular, anatomical), to the highly abstract and theoretical one. The first step in understanding the structure/function relationships in BSR involves isolating the components of the system that underlie this behaviour (basic level). The second step is the construction of theoretical models to make sense of the interrelationships between these components, synthesizing them into a global framework (theoretical level) (Stellar and Stellar, 1985).

In this section, we first address the two basic levels of analysis: cellular and anatomical, followed by a brief synthesis of some theoretical models that have been proposed to account for the phenomenon of BSR. Because the research presented in this dissertation is situated at an anatomical level of analysis - exploring the amygdaloid complex as a likely candidate to participate in the MFB reward circuit - most emphasis is placed in developing this aspect. In this context, when extra-MFB sites are considered, please note that the link between the MFB and the amygdaloid complex will not be mentioned until the second section (the amygdaloid complex) of

this introduction.

1.4.1 Basic levels of analysis

1.4.1.1 Cellular level: psychophysical data

Data from psychophysical techniques have contributed to clarifying some of the functional characteristics of the neural substrate underlying BSR. One such method is that of the determination of refractory periods. They can be assessed in an individual axon bundle by eliciting different profiles of action potentials via a manipulation of the interval between two pulses applied to this axon bundle (Stellar and Stellar, 1985). The physical attributes of axons, such as their size and their myelination, determine the length of their refractory periods, which thus inform us about their biological nature.

It has been shown that the refractory period of the neurons directly activated by the electrode in BSR ("first-stage" neurons) range between 0.4 to 1.3 milliseconds (Yeomans, 1975). Thus, these cells are most probably myelinated, small in diameter (.3-1.5 μ), and conduct impulses at velocities of 2-8 m/s (Bielajew and Shizgal,1980, 1982). Interestingly, this finding refutes the idea that dopaminergic fibers are the directly excited substrate of BSR since the latter are unmyelinated and have refractory periods that vary between 1.8 and 2.0 milliseconds (German, Dalsass, and Kiser, 1980; Guyenet and Agajanian, 1978; Yeomans, Maidment, and Bunney, 1988).

1.4.1.2 Anatomical level: the median forebrain bundle

At an anatomical level of analysis, the MFB is considered to be a primary substrate for BSR due to the ease with which one can obtain this behaviour as a consequence of stimulation. Acquisition of the operant response does not take more than a few minutes, and the self-administration of the stimulations is exuberant, with bar-pressing rates habitually above 60/min (Yeomans, 1990). Over the past several decades, much effort has been invested in characterizing the intrinsic anatomical map, the inputs to and the outputs from this fiber bundle. Anatomical data reveals that the MFB is made up of as many as 50 thin descending and ascending fibers of different lengths, origins and destinations (Nieuwenhuys, Geeraedts, and Veening, 1982; Veening et al., 1982) (see Appendix 1).

A unique opportunity to explore anatomical regions activated by BSR was provided by Kennedy et al.(1975) and Sokoloff et al.'s (1977) development of the 2-deoxyglucose (2-DG) method to measure regional brain metabolism. This method is based on the principle that when the brain is physiologically activated, it requires a considerable amount of energy that is provided on a short-term basis by glucose consumption (Stellar and Stellar, 1985). The analog of glucose, 2-DG, can be radioactively labelled and tracked throughout the brain.

A number of investigators (Gallistel, Karreman, and Reivicc, 1977; Yadin, Guarini, and Gallistel, 1983; Yeomans et al., 1975;) used this technique to map out the general anatomical substrate directly activated by the stimulating electrode in BSR. Based on this collective effort, Gallistel (1983) subsequently generated a computer-assisted 2-DG map of brain activation related to MFB self-stimulation. His work revealed that both anterior and posterior MFB self-stimulation sites produce similar patterns of brain activation that follow along the whole length

of the MFB and terminate in the VTA (see Stellar and Stellar, 1985).

Following this demonstration that BSR produces metabolic brain activation from the LH to the VTA (Kennedy et al., 1975; Sokoloff et al.,1977), the question of anatomical linkage between these two sites became an important one at the anatomical level of analysis. The most convincing way to demonstrate that two electrodes are located on the same axonal pathway is via the collision method. Succinctly, this method is based on a basic neurobiological property that axonal propagation of impulses is bidirectional so that action potentials that conduct toward each other collide (see Stellar and Stellar, 1985). Thus, if two pulses are simultaneously delivered via two stimulating electrodes implanted on the same pathway, action potentials travelling orthodromically will be cancelled out when meeting action potentials travelling in the opposite direction (antidromically) (for a more detailed description of this technique, see Bielajew and Shizgal, 1982, and Miguelez and Bielajew, In Press) Using this method, Bielajew and Shizgal (1980, 1982) showed that the LH and VTA share common fibers.

Metabolic approaches such as the one described earlier have also been employed to investigate sites located outside of the MFB that may contribute to the rewarding effect of MFB stimulation. Recall that this idea has anatomical validity, since the MFB comprise more than 50 different fiber systems which follow distinct trajectories (see Nieuwenhuys, Geeraedts, and Veening, 1982; Veening et al., 1982). Additionally, and as previously mentioned, BSR can be obtained from numerous sites other than in the MFB, including regions as anterior as the olfactory bulb to as posterior as the medulla (Yeomans, 1990).

Esposito et al. (1984) are one group among many (Gallistel et al., 1985; Porrino et al., 1984; 1990; Simmons, Ackerman, and Gallistel, 1998) who used the 2-DG method to

demonstrate that rewarding stimulation evoked from within the MFB elicits an increase of short-term metabolic activity outside of this region. In their study, they showed that BSR elicited from the VTA of freely moving rats activates a vast number of extra-MFB regions, in rostral (e.g. the preoptic area), forebrain (e.g. the basolateral and central amygdaloid nuclei), and caudal locations (e.g. the dorsal raphe). Simmons, Ackerman, and Gallistel (1998) confirmed that VTA self-stimulation increases 2-DG activity in the medial preoptic area, as well as in the fundus of the striatum and the nucleus of the diagonal band. The latter region was also metabolically activated following posterior MFB self-stimulation in Gallistel et al. 's (1985) study. Porrino and her group (1984, 1990) found that VTA self-stimulation provoked marked elevations of 2-DG activity in more than thirty extra-MFB sites, including many regions contralateral to the stimulating electrode, suggesting a bilateral involvement of a wide array of ascending and descending systems.

Another metabolic method more recently employed to track brain regions associated with BSR is that based on the measurement of cytochrome oxidase. This enzyme participates in the mitochondrial respiratory chain which generates energy by oxidative phosphorylation (Nicholls and Butko, 1993). Since neurons derive their metabolic energy using almost exclusively the aerobic pathway, ion pumping is related to the mitochondrial gene expression. In fact, high levels of cytochrome oxidase activity have been correlated with brain areas of high functional activity (Hevner, Liu, and Wong-Riley, 1995; Hevner and Wong-Riley, 1993). Thus, although neurons are dependent on glucose as their primary energetic substrate (short-term metabolic demands), oxidative phosphorylation is the primary pathway to generate adenosine triphosphate (longer-term metabolic demands). These two techniques can be used to track metabolic activity

taking place over different time periods and along metabolic pathways that, although not mutually exclusive, do not completely overlap (see Harley and Bielajew, 1992; Hevner, Liu, and Wong-Riley, 1995).

Although the technique of cytochrome oxidase histochemistry was originally designed to track the functional consequences of sensory deprivation on specific regions of the brain (see Wong-Riley, 1989; Wong-Riley and Carroll, 1984), Bielajew and her group (Bielajew, 1991; Bielajew and Fouriezos, 1986; Harley and Bielajew, 1992; Sandner, Bielajew, and Fouriezos, 1996) showed its exceptional usefulness to the exploration of the BSR pathway in a series of elegant studies. Most relevant to the present discussion is their finding that mesocorticolimbic sites exhibit an increased level of cytochrome oxidase activity following LH self-stimulation, suggesting their involvement in modulating MFB reward (Bielajew, 1991).

Lesion techniques are yet another tool exploited in an effort to characterize the anatomical map underlying MFB-derived BSR. Within the MFB, a series of early investigations failed to alter BSR by destroying sites anterior to the self-stimulation electrode (see Lorens, 1976). The few exceptions (Boyd and Gardner, 1967; Morgane and Kosman, 1960) that showed effects of such lesions are difficult to interpret given their use of response rates as a measure of reward (see section 1.3 of this document). In those cases, a reduction of performance could be related to motoric or other non-reward factors instead of reflecting an alteration of the rewarding value of the stimulation per se (also see Stellar and Stellar, 1985). Conversely, lesions of the MFB posterior to the stimulation site are thought to be more effective in altering BSR derived from the MFB. For instance, Gallistel et al. (1996) compared the efficacy of lesions made either anterior or posterior to the stimulating electrode. They found that the latter type of damage

reduced the rewarding efficacy of the MFB stimulation by 35-60%, whereas anterior lesions barely affected the behaviour. It should be noted that this group obtained very similar results whether using lesions induced be electrolytic or knife-cut methods.

As with metabolic studies, lesion techniques also contributed in revealing the involvement of extra-MFB sites to the rewarding effect of MFB stimulation. Although a detailed review of such studies is beyond the scope of this dissertation, it should be mentioned that this exploration has focussed on regions as varied as the central gray (Waraczynski and Perkins, 1998), dorsal raphe (Waraczynski and Perkins, 1998), parabrachial nucleus (Waraczynski and Shizgal, 1995), preoptic area (Arvanitogiannis, Waraczynski and Shizgal, 1996), and motor nucleus of the trigeminal nerve (Acheson, Waraczynski, and Perkins, 2000), just to name a few.

Regardless of the lesion technique, site, or stimulation placement, the common thread between all these studies appears to be a lack of consistency in the impact of the lesions on MFB reward. Across investigations and even within the same studies, lesions typically have very different effects on reward thresholds (see Appendix 2). For instance, Acheson, Waraczynski, and Perkins, (2000) tracked the influence of serial bilateral electrolytic lesions of the trigeminal nerve on LH and VTA reward thresholds. At the VTA site ipsilateral to the lesion, one rat showed an increase in the rewarding value of the stimulation whereas a second animal exhibited exactly the opposite effect. At the LH site ipsilateral to the lesion, four animals showed no post-lesion effect whereas one rat exhibited a moderate increase in the rewarding value of the stimulation.

These apparent contradictions actually validate the logic underlying the use of lesion techniques in the search for the substrate of BSR elicited from the MFB. Such models are based

on the premise that the destruction or inactivation of a relevant part of the reward pathway should result in an alteration of BSR, as operationally defined by post-lesion modifications of reward thresholds. Conversely, if large lesions fail to affect the rewarding value of the stimulation, one can assume that the affected pathways are not implicated in the behaviour. Particularly informative in delineating potential pathways are the cases in which very effective lesions are shown in combination with ones that have no apparent behavioural effect (Stellar and Stellar, 1985), since a clearer idea of the anatomical boundaries of pathways relevant to the target behaviour can then be achieved. Thus, careful application and interpretation of lesion techniques allows some insight into the mapping of structures that are crucial in modulating the MFB reward signal.

1.4.2 Theoretical level of analysis

1.4.2.1 The minimal model and the descending path hypothesis

The minimal model of Gallistel, Shizgal, and Yeomans (1981) attempts to lay down some basic yet abstract assumptions to account for the phenomenon of BSR. Their conception of the "reward pathway" begins with a cable-like substrate that represents the directly excited reward-related axons (also called "first-stage reward neurons"). As the action potentials evoked in this cable travel across a complex maze of neurons and synapses, their effects summate temporally and spatially within a second hypothetical substrate called the "integrator". Following the integrator's activity, a conversion takes place in order to code and keep a memory of the subjective intensity of the reward - this memory is called an engram. The final output (behaviour) resulting from this process depends on the summed effects of three variables: the

engram, the priming effect (a brief strengthening of the behaviour rewarded by brain stimulation), and the influence of other performance factors (such as the health of the organism).

One such reward pathway as the one envisioned in the minimal model is consistent with the converging lines of evidence from lesion, metabolic, and psychophysical investigations (see above). Informally labelled the "descending path hypothesis" (Stellar and Stellar, 1985), this model submits that the basic reward pathway originates in an unknown site outside of the MFB, courses into and/or through the VTA, and that the axons that make up this pathway are small and myelinated. The loci of origin of such a hypothetical pathway has been the subject of much speculation in the last decades. If one embraces this model, one likely candidate is the amygdaloid complex, for reasons described in the next section of this introduction.

1.4.2.2 The model of collateralization

An alternative theory to the minimal model and the descending path hypothesis has recently been submitted by Gallistel and his collaborators (1996). This idea stems from their demonstration that although VTA lesions can elicit robust decreases in the rewarding value of LH self-stimulation, LH damage causes much more erratic consequences on BSR of the VTA and/or of more dorsal LH sites (Gallistel et al., 1996). The investigators propose that their data are incompatible with the notion that reward fibers course together in a compact bundle that either descends or ascends in or near the MFB. If the descending hypothesis were true, lesions to the MFB anterior to the stimulation site would consistently disrupt the rewarding value of the stimulation.

Conversely, an alternative scenario is that the reward fibers are very collateralized in that

their topography rather resembles a net-like arrangement rather than of one akin to a cable. The reward neurons are conceived as being bipolar with both their somata and their terminals located in the midbrain. The investigators (Gallistel et al., 1996) further suggest that these fibers are distributed over an ample region that probably extends beyond the limits of the MFB. Thus, lesions to the MFB anterior to the stimulation site will leave a preponderance of fibers untouched, having little or no effect on the rewarding value of the stimulation. Conversely, the superior effectiveness of lesions located posterior to the stimulation site is interpreted as an indication that the reward fibers are more clustered in the caudal hypothalamus and anterior VTA than in the more anterior hypothalamus.

This theory has been questioned by Shizgal (1997), on the basis of its inability to explain the numerous cases in which MFB lesions placed anterior to the stimulating electrode significantly reduce the rewarding value of the stimulation. Furthermore, the model does not take into account that damage to VTA dopaminergic neurons may have been an important factor in the emergence of these lesion effects. Notwithstanding these limitations, the idea is creative and like the descending path hypothesis, encourages an exploration of extra-MFB sites as potential participants in the substrate for MFB reward. The potential contribution of the amygdaloid complex in this circuit is the object of the research presented in this dissertation.

2. THE AMYGDALOID COMPLEX

2.1. OVERVIEW OF THE LIMBIC SYSTEM AND OF THE AMYGDALA

"*The [limbic system] had developed 150 million years ago, when reptiles ruled the earth. It controlled the most primitive behavior - anger and fear, lust and hunger, attack and withdrawal. Reptiles like crocodiles had little else to direct their behavior. Man, on the other hand, had a cerebral cortex. But the cerebral cortex was a recent addition. Its modern development had begun only two million years ago; in its present state, the cerebral cortex of man was only 100,000 years old. In terms of evolutionary time scales, that was nothing. The cortex had grown up around the limbic brain, which remained unchanged, embedded deep inside the new cortex. That cortex, which could feel love, and worry about ethical conduct, and write poetry, had to make an uneasy peace with the crocodile brain at its core*" Crichton, M. (1972) p. 156.

When separating the brain into its two hemispheres, the medial divisions of the frontal, parietal, and temporal lobes form a continuous body of cortex that lies over the rostral brain stem and diencephalon. As early as 1937, a neuroanatomist by the name of Papez introduced the idea that these structures formed a circuit involved in motivation and emotion (Carlson, 1994). A few years later, MacLean expanded on this concept, adding that the emergence of emotional responses appeared to correspond with the evolution of this circuit which he called "the limbic system" (Carlson, 1994). Today, it is well-established that the neurons of the limbic system form a network of circuits that unitedly play an important role in learning, memory, and emotions (Kandel, Schwartz, and Jessell 1991). Within the medial walls of the temporal lobes lies the

amygdaloid complex or region, a key component of the limbic system of the brain. Because of its elongated shape, the name amygdala, originating from the Greek word for "almond", has been given to this structure (see Figure 3) (Aggleton, 1992).

Figure 3. This figure schematically shows the location of the amygdaloid complex in a sagittal section of the rat brain. Note that its shape is elongated, reminiscent of that of an almond.

Although the brain of all vertebrates has an amygdala, the areas supplying sensory information to this region are thought to have changed over the course of evolution. In non-mammalian species, which have diminutive or no cerebral cortex (e.g. crocodiles), the main sources of olfactory and non-olfactory projections to the amygdala are respectively the olfactory bulb and diencephalon (Bruce and Neary, 1995). The results of comparative neuroanatomical studies suggest that the mammalian evolution of the cerebral cortex gradually transformed it into the most important source of sensory information to the amygdala. Furthermore, the evolutionary development of the mammalian cortex corresponds to the growth and differentiation of the amygdala (Stephan and Andy, 1977, in McDonald, 1998). These considerations, coupled with the results of countless investigations over the course of the last century, confirm this locus as being a cerebral site of major importance in processes as various as those involved in learning, memory, motivation and emotion (McDonald, 1998). In order to understand these processes, all of which are pivotal to human and animal behaviour, the study of the amygdaloid region and of its detailed workings and interconnections with other sites of the brain is a fundamental step towards this goal.

2.2. THE AMYGDALOID SUB-NUCLEI

"The amygdaloid complex remains an enigma" Amaral et al. (1992) p.1.

2.2.1 Basis of amygdaloid terminology and nomenclature

In all mammals, the anatomy of the amygdaloid region is extremely complex, containing a myriad of nuclei that often merge with each other, as well as with neighbouring non-amygdaloid regions (McDonald, 1998). Because this area is made up of at least ten distinct nuclei and cortical areas, many of which are further divided into subregions (Price, Russchen, and Amaral, 1987), the term "amygdaloid complex" has progressively taken over the more unadorned name of "amygdala". The heterogeneous group of nuclear and cortical structures that comprise the amygdaloid complex are usually differentiated on the basis of their cellular architecture and histochemical characteristics (Amaral et al., 1992; Price, 1973; Price, Russchen, and Amaral, 1987).

Although the cytoarchitectonic organization of the amygdaloid complex has been studied for about a century (Volsch, 1906, 1910, in Price, Russchen, and Amaral, 1987), both the terminology and delimitation of its nuclei remain equivocal. One reason for this disagreement is the existence of two major schools of thought regarding the conceptualization of the amygdaloid complex. The first, embraced by Japanese investigators such as Uchida (1950, in Price, Russchen, and Amaral, 1987) and Koikegami (1963), adopted the German legacy of Volsch (1906, 1910 in Price, Russchen, and Amaral, 1987) and divided the amygdaloid complex into several small subdivisions. In contrast, American and European researchers have generally

followed the nomenclature originally suggested by Johnston (1923), which is based on a first subdivision of the amygdaloid complex into fewer major nuclei, some of which are further classified into two or three parts (Price, Russchen, and Amaral, 1987).

The result has been that both groups have used different terms to name seemingly homologous regions in different or even in the same species. For instance, the region designated "basal nucleus" in the primate brain (Crosby and Humphrey, 1941, 1944) is usually known as the "basolateral nucleus" in the cat and rat. Similarly, the cells contained within the accessory basal nucleus of the primate appear to be comparable to those in the basomedial nucleus of rodents (Price, Russchen, and Amaral, 1987). Also, in the case of the rat, MacDonald's (1998) basal and accessory basal nuclei correspond to Paxinos and Watson's (1986, 1998) basolateral and basomedial nuclei respectively (these equivalencies in terminology are further elaborated in section 2.2.2 of this document as well as in Appendix 3).

On addition to these discrepancies, the terminology has evolved through three general eras. Originally, the determination of anatomical boundaries in brain tissue was simply based on a visual analysis of the amygdaloid nuclei in Nissl-stained material (Price, Russchen, and Amaral, 1987). A major improvement in nomenclature was subsequently achieved with the development of histochemical techniques, primarily that of the identification of acetylcholinesterase (AChE), which was able to confirm certain boundaries such as that of the basal nucleus in the primate brain and the basolateral nucleus in the feline and rodent brain (Price, Russchen, and Amaral, 1987).

The histochemical technique to visualize AChE, the acetylcholine (ACh)-degrading enzyme present in cholinergic neurons, was first developed by Koelle and Friedenwald (1949),

and further elaborated by Karnovsky and Roots (1964) a decade and a half later. The neurotransmitter ACh consists of choline and of an acetyl group produced intracellularly by oxidative metabolism in mitochondria. Because the synthesis of ACh takes place in the axonal terminals and is catalyzed by the cytosolic enzyme choline acetyl transferase, this immunocytochemical approach in neural mapping has the ability to provide exquisite details at the levels of individual cells (Van der Zee and Luiten, 1999).

In the third epoch of the evolution in amygdaloid terminology, the definition of nuclear boundaries was further refined by combining the existing cytoarchitectonic, histochemical and immunohistochemical information with newer data regarding connections. For instance, Hall (1972) showed that the basolateral amygdaloid nucleus is comprised of two major types of neurons: spiny pyramidal and spine-sparse non-pyramidal ones. The non-parallel arrangement of these neuron's dendrites appears to be a distinguishing characteristic of the basolateral nuclei of all species investigated to date (McDonald, 1998).

It should be pointed out that in general, there is no clear set of rules to categorize the components of the nervous system, so that these can therefore be quite arbitrary (Isaacson, 1982). The value of having guidelines to categorization is that it aids us in understanding brain activity and how it relates to behaviour (Isaacson, 1982). The integration of data about connections in amygdaloid nomenclature has enabled investigators to a achieve a deeper understanding of the basis and functional anatomy of the amygdaloid complex.

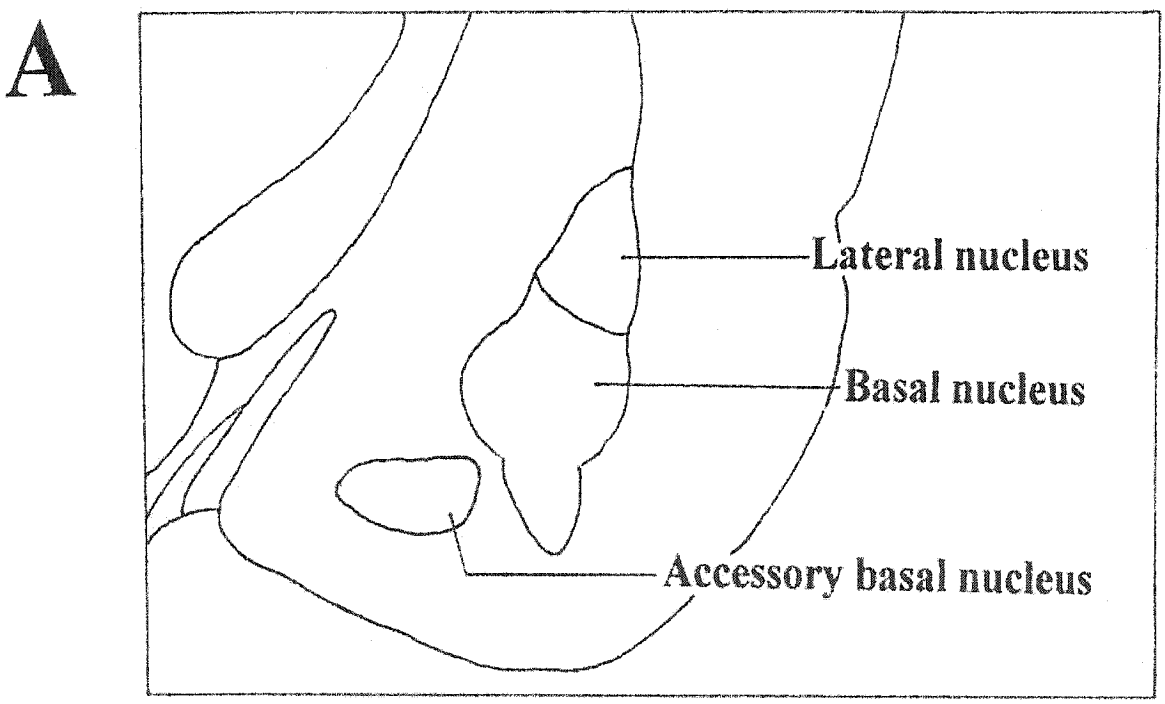
2..2.2 Cell groups of the amygdaloid complex

At present, the North-American and European literature most commonly divides the amygdaloid complex into: (1) the basolateral nuclear group, which comprises the lateral, basal and accessory basal nuclei - profusely interconnected with the neocortex, (2) the corticomedial nuclear group (also often referred to as the superficial cortex-like nuclear group - see McDonald, 1998), which include the anterior and posterior cortical nuclei, the bed nucleus of the accessory olfactory tract, and the periamygdaloid complex, and (3) the centromedial nuclear group, comprising the medial and central nuclei - sharing connections with the autonomic control regions of the lateral hypothalamus and brainstem (McDonald, 1998; Price, Russchen, and Amaral, 1987). Although some anatomists also include the bed nucleus of the stria terminalis in the centromedial group (Paxinos and Watson, 1986), others rather view this region as being a rostral extension of these nuclei (McDonald, 1998). These subdivisions are illustrated in Figure 4.

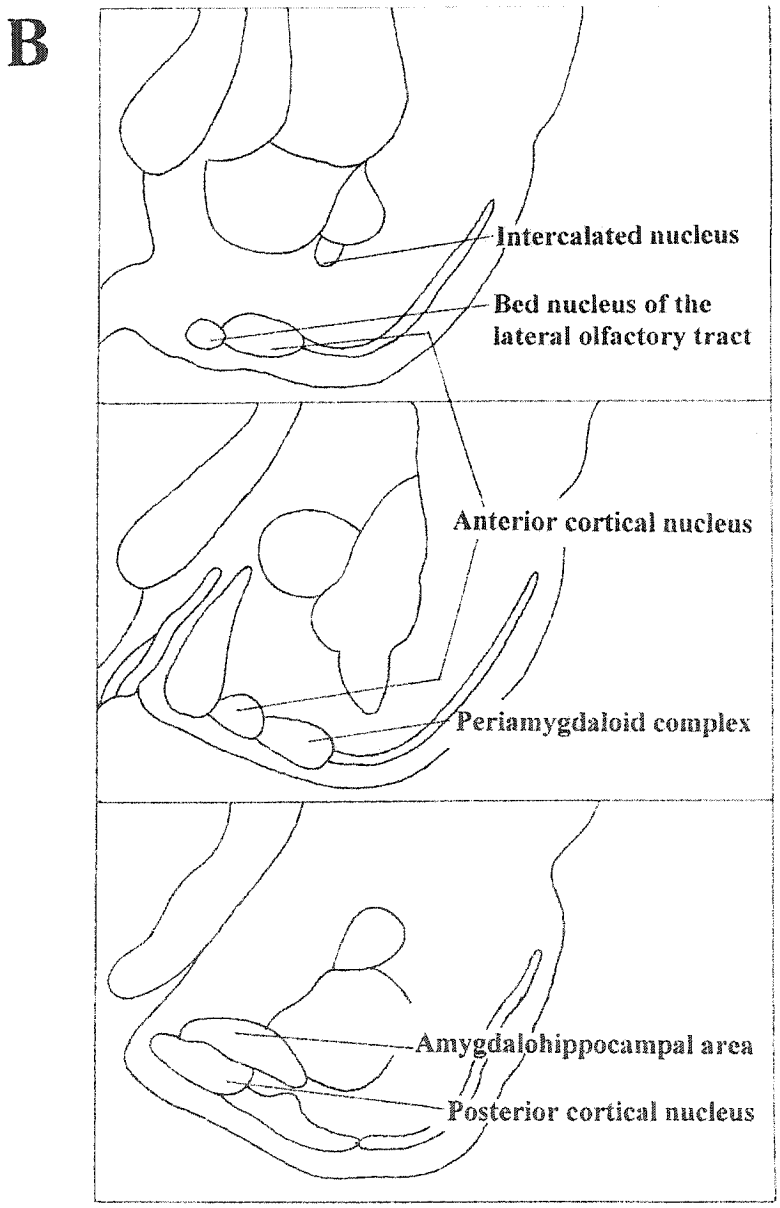
As pointed out earlier, this terminology can be somewhat confusing since investigators have used different labels to name homologous regions. Most relevant in this report, equivalencies between McDonald (1998) and Paxinos and Watson's (1986, 1998) terminologies should be pointed out: respectively, the basal corresponds to the basomedial nucleus, the accessory basal to the basomedial nucleus, the posterior cortical to the posteromedial nucleus, the accessory olfactory tract to the lateral olfactory tract, and the periamygdaloid complex to the posterolateral subdivision of the cortical nucleus (also see Appendix 3). In the present account, it should also be noted that only the nomenclature of the amygdaloid complex as applied to the rat brain will be considered, with occasional comparisons to the cat and primate brain.

Figure 4. This figure depicts the organization of the four major amygdaloid nuclear groups: Basolateral, Corticomedial, and Centromedial, and Other, as defined by McDonald (1998) in a series of coronal sections corresponding three anterior-posterior levels defined by Paxinos and Watson (1998) .

A shows the three components of the Basolateral nuclear group: the Lateral, Basal, and Accessory basal nuclei. This section corresponds to the anterior-posterior level located at -2.80 mm behind bregma.

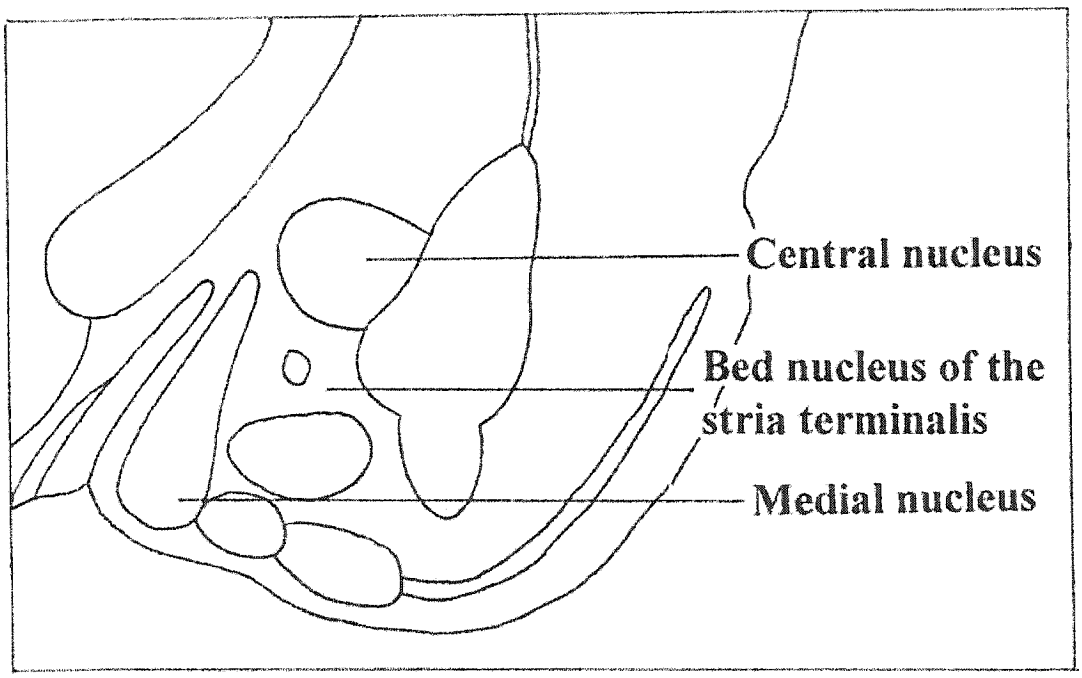


B illustrates the four components of the Corticomedial nuclear group and the two components of the Other nuclear group. In the top panel, the bed nucleus of the lateral olfactory tract is shown in relation to the anterior cortical nucleus and to an intercalated nucleus (the latter is classified in the "other" amygdaloid nuclear group) in a section corresponding to the anterior-posterior level located at -1.80 mm behind bregma. In the middle panel, the anterior cortical nucleus and periamygdaloid complex are illustrated in a section corresponding to level -2.80 mm behind bregma. In the bottom panel, the posterior cortical nucleus is shown in relation to the amygdalohippocampal area (the latter is classified in the "other" amygdaloid nuclear group), at a level located at -3.60 mm behind bregma.



C depicts the three components of the Corticomedial nuclear group in a section corresponding to level -2.80 behind bregma: the Medial and Central nuclei as well as the Bed nucleus of the stria terminalis.

C



2.2.2.1 The basolateral nuclear group

A. The lateral nucleus

In all the species evaluated to date, the lateral nucleus is the largest one of the amygdaloid complex, located throughout its entire rostro-caudal extent (Isaacson, 1982). As indicated by its name, this nucleus is the most lateral component of the basolateral group not only in the rat, but in the cat and monkey as well (McDonald, 1998). On its lateral flank, it touches the external capsule, and on its medial aspect, it is bordered by the longitudinal association bundle. As compared to the basal nucleus, which is located ventral to it, the cells of the lateral nucleus are smaller in size and contain lower concentrations of AchE (Price, Russchen, and Amaral, 1987).

B. The basal or basolateral nucleus

In most species, the largest cells in the amygdaloid complex are contained within the basal (originally called basolateral) nucleus. Because of this cellular hallmark, it can be easily distinguished from the lateral nucleus, which is located immediately above it (Isaacson, 1982; McDonald, 1998). The basal nucleus has the highest concentrations of AchE and Choline acetyltransferase of the entire amygdaloid complex (Ben-Ari et al., 1977; Price, Russchen, and Amaral, 1987). Spanning throughout nearly all of the rostrocaudal breadth of the complex, it is divided into two sections that are differentiated by cell size and content of the cholinergic marker enzymes (Price, Russchen, and Amaral, 1987). The magnocellular division, also termed the anterior basolateral nucleus, lies rostral and medial to the parvicellular division, also known as the posterior basolateral nucleus (Krettek and Price, 1978 b).

C. The accessory basal or basomedial nucleus

The accessory basal nucleus, originally known as the basomedial nucleus, is located in the caudal half of the amygdaloid complex, beneath the basal nucleus and dorsal to the periamygdaloid cortex (Isaacson, 1982), and is distinguished as a lightly staining structure when treated with AchE (Price, Russchen, and Amaral, 1987). The cells in the rostral and medial parts of this nucleus are generally bigger than those in the caudolateral zone, hinting at a similarity with the magnocellular division of the monkey accessory basal nucleus (Price, Russchen, and Amaral, 1987).

2.2.2.2. The corticomedial nuclear group

This nuclear group deserves a special foreword, not only because it is located at the surface of the brain, but also since each of its components comprises two very distinct molecular layers. The first, called layer I, is located outwardly and is characterized by its scarce cells which also contain fibers from the main and accessory olfactory bulbs. The deeper-dense zone of these nuclei, termed layer II, has a laminar arrangement (McDonald, 1998).

A. The anterior cortical nucleus

The anterior cortical nucleus of the rat begins rostrally directly lateral to the nucleus of the lateral olfactory tract and faintly stains for AChE and Choline acetyltransferase. Comparable to the monkey, it is composed of two layers of small and medium-sized cells, with the deeper ones forming an oval mass that is easily distinguished microscopically (Isaacson, 1982).

B. The posterior or posteromedial cortical nucleus

Although many investigators only identify an anterior partition to the cortical nucleus, Paxinos and Watson (1986) propose a posterior subdivision, which they call the "posteromedial cortical nucleus". It is the most caudal component of the entire superficial corticomedial nuclear group (McDonald, 1998), located medial and caudal to the periamygdaloid cortex. This subdivision contains a homogenous cluster of cells arranged in a rounded fashion. When treated with AChE, its pale stain contrasts with the darker amygdalo-hippocampal area located dorsal to it (Price, Russchen, and Amaral, 1987).

C. The bed nucleus of the accessory or lateral olfactory tract

Although some of this nucleus's small cells are disseminated all along the trajectory of the accessory olfactory tract, most of them merge into a nucleus in the rostral part of the amygdala. From this point, the nucleus extends into layer I between the nucleus of the lateral olfactory tract and the medial nucleus. Alike the medial and posterior cortical nuclei, when treated with AChE, a dark band of staining material can be seen in the superficial part of layer I (Price, Russchen, and Amaral, 1987). Interestingly, although the bed nucleus of the accessory olfactory tract has been identified not only in the rat, but also in the rabbit and opossum (Scalia and Winans, 1975), a homologous structure does not seem to exist in the primate brain (Price, Russchen, and Amaral, 1987), which may suggest a lesser reliance on olfaction in higher species.

D. The periamygdaloid cortex or posterolateral subdivision of the cortical nucleus

To date, most studies describe the periamygdaloid cortex as a somewhat large, heterogeneous stretch of cells on the ventral surface of the amygdaloid complex (Price, Russchen, and Amaral, 1987), with a visible thin but compact layer I and a slightly thicker and less developed layer II (Isaacson, 1982). In most species, it is located laterally to the piriform cortex, medially to the anterior cortical nucleus and amygdalo-hippocampal area, and caudally to the entorhinal cortex. At the caudal extremity of this cortex, the cortical layers merge with the caudal tip of the paravicellular basal nucleus, into what is termed the sulcal region (Price, Russchen, and Amaral, 1987).

2.2.2.3 The centromedial nuclear group

A. The medial nucleus

In the rat brain, the medial nucleus is exceptionally well delineated, clearly visible at the level of the medial extremity of the rostral two-thirds of the whole amygdaloid complex (Price, Russchen, and Amaral, 1987), just lateral to the preoptict tract (McDonald, 1998). In aspect and diameter, the cells of this area are small and resemble those in the anterior cortical nucleus (Isaacson, 1982), with the difference that they are arranged in a much more compact manner, allowing for a clear differentiation between the two nuclei (Price, Russchen, and Amaral, 1987). Although the medial nucleus does not stain well for AChE, the Timm method of staining clearly delineates the boundary between the projections of the main and accessory olfactory bulbs (De Olmos, Alheid, and Beltramino; Friedman and Price, 1984).

At the rostral and caudal extremities of the medial nucleus, two areas can be distinguished

from the main body of the nucleus. The first, located at the level of the rostroventromedial corner of the nucleus, can be distinguished by its small compactly arranged cells and is known as the rostral subdivision. This zone lies directly medial to the anterior cortical nucleus and is gives rise to the largest source of medial nucleus projections to the ventromedial hypothalamus (Wiegand and Price, 1980). To our knowledge, a region comparable to the rostral subdivision has not been characterized in the monkey and cat amygdaloid complexes.

The caudal subdivision of the medial nucleus is larger than the rostral one, comprising the caudal third of the entire medial nucleus (Price, Russchen, and Amaral, 1987) and merging with the posterior cortical nucleus and the amygdalohippocampal area (Isaacson, 1982). Cytoarchitectonically, it is characterized by cells which are more densely arranged than those of the main body of the nucleus, and connectively, it is circumscribed by a clearly visible bundle of fibers that is continuous with the stria terminalis (Price, Russchen, and Amaral, 1987).

B. The central nucleus

In the rat and monkey brain, the central nucleus takes up a substantial proportion of the caudal amygdaloid complex. It is located immediately lateral to the medial nucleus (McDonald, 1998). On the basis of Nissl and AChE-stained tissue appearance, medial and lateral sub-divisions can be distinguished in this nucleus (Isaacson, 1982; Price, Russchen, and Amaral, 1987); furthermore, in the last decade, four different sub-divisions of this nucleus have been identified from Nissl and Golgi preparations in the rat brain (McDonald, 1982). The first, called the medial division of the central nucleus, is located mostly in the rostral half of the central nucleus and is constituted by somewhat large, non-compressed cells and some AChE-stained

fibers. The second sub-division, located in the lateral part of the nucleus, contains smaller and more compactly arranged cells and has a circular shape that surrounds the medial side of the lateral and basal nuclei. This region does not stain well for AChE and can therefore be differentiated easily from both the medial division of the nucleus and the laterally contiguous basal and lateral nuclei (Price, Russchen, and Amaral, 1987). The fourth component of the central nucleus, described by McDonald (1998) as the "lateral capsular division", constitutes simultaneously the dorsolateral apex of the lateral division and its lateral frontier with the basal nucleus. The apex corresponds to a fragment of the putamen which has been designated "PX" by Price, Russchen, and Amaral (1987), whereas the ventrolateral portion is a segment of the intercalated nuclei named the "amygdalostriatal transition zone" by De Olmos, Alheid, and Beltramino (1985).

In the last two decades, it has been recognized that the dorsal portion of the central nucleus of the amygdala elongates rostrally towards the bed nucleus of the stria terminalis. These two nuclei and the cell bridges situated in the sublenticular region dorsal and rostral to the central nucleus demarcate the region called "the extended amygdala", described in more detail in section 2.3.2.4 of this document) (Alheid and Heimer, 1988).

C. The bed nucleus of the stria terminalis

The bed nucleus of the stria terminalis (BNST) is sub-divided into two parts which are separated by the commissural bundle of the stria terminalis: the medial portion is characterized by cells that are smaller and more compactly arranged than that of the lateral division (Price, Russchen, and Amaral, 1987).

2.2.2.4 Other amygdaloid nuclei

A. The amygdalohippocampal area

This area lies between the caudal extremity of the amygdaloid complex and the ventral subicular complex of the hippocampal formation. Darkly staining for AChE, it is made up of medium-sized cells that become compactly arranged at the point at which they become connected with the pyramidal cell layer of the subiculum (Isaacson, 1982; Price, Russchen, and Amaral, 1987).

B. The intercalated nuclei

The intercalated nuclei are small groups of compactly arranged neurons which are associated with the fiber bundles that separate the amygdaloid nuclei (McDonald, 1998). They are most evident between the basal nucleus and the anterior cortical as well as rostroventrally to the anterior amygdaloid area (Price, Russchen, and Amaral, 1987).

2.2.3 Intrinsic intraamygdaloid connections

In the decade of the 1960's, investigators using axonal degeneration methods began establishing the elemental outline of intraamygdaloid connections (Price, Russchen, and Amaral, 1987). More detailed information was progressively added to this knowledge through the use of various techniques, namely the anterograde or retrograde axonal transport (most extensively used in the study of intrinsic intraamygdaloid connections), the histochemical and immunohistochemical methods, and the use of metabolic tracers such as 2-deoxyglucose (Price, Russchen, and Amaral, 1987).

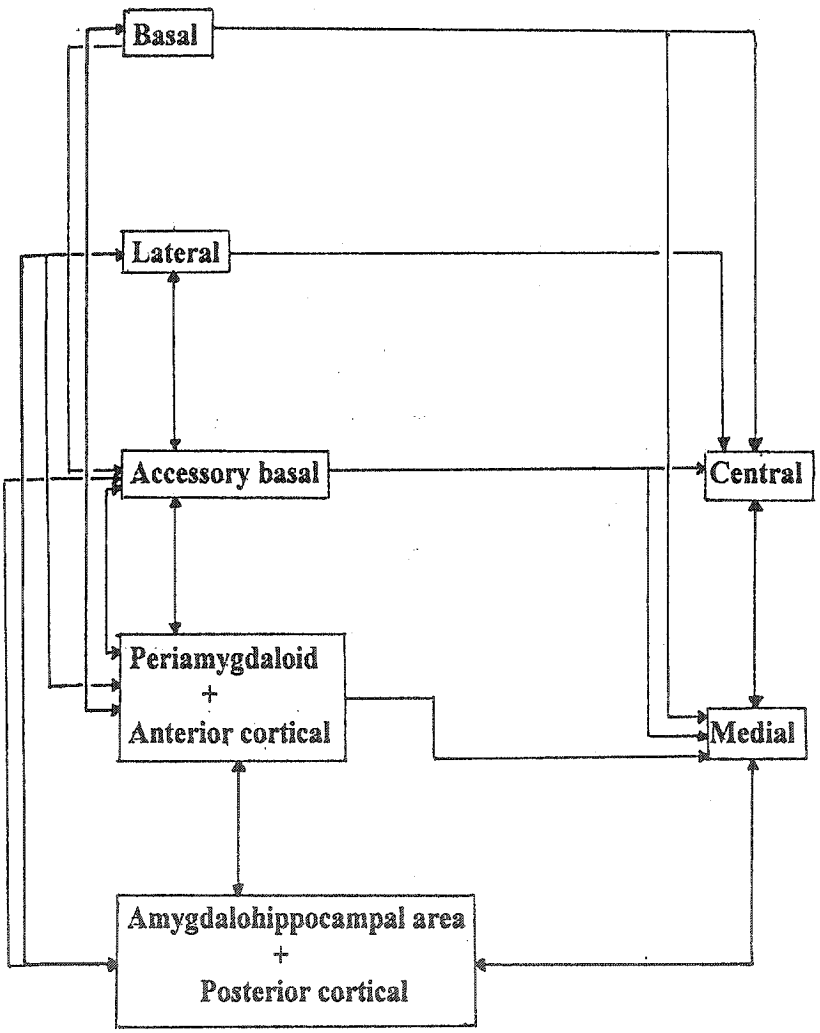
The complex arrangement of intrinsic connections between amygdaloid nuclei is schematically represented in figure 5 in relation to their cytological configuration. This circuitry allows the amygdala to integrate incoming messages from some of the highest brain centers, thus influencing the subsequent amygdaloid output to the various regions modulating endocrine, visceral, affective, and mnemonic processes (McDonald, 1992).

In general, the projections of the deeper amygdaloid nuclei (illustrated on the top part of figure 5) to the superficial ones (on the bottom of figure 5) are more substantial than the projections in the opposite direction, although one notable exception to this arrangement is the lack of connections between the lateral and basal nuclei (McDonald, 1992). The deeper nuclei appear to project mainly to the central nucleus, whereas the superficial nuclei project primarily to the medial nucleus. This distinction is especially meaningful since the central and medial nuclei influence different subcortical structures via their efferent projections (McDonald, 1992). It has been firmly established that the most predominant intraamygdaloid connections originate in the lateral and basal nuclei and end in the more medially located nuclei, notably the accessory basal, central, medial and anterior cortical nuclei, and the periamygdaloid cortex as well as the amygdalo-hippocampal area. Additionally, the accessory basal nucleus sends efferents to the central, medial and cortical nuclei and to the amygdalo-hippocampal area (Price, Russchen, and Amaral, 1987).

This arrangement of interconnections suggests that the sensory information (such as visual, auditory, and somesthetic) originating from sensory association cortices is transmitted through the intra-amygdaloid connections from the basolateral nuclei to the olfactory receiving zones of the superficial amygdala. The periamygdaloid and accessory basal nuclei may be

through the intra-amygdaloid connections from the basolateral nuclei to the olfactory receiving zones of the superficial amygdala. The periamygdaloid and accessory basal nuclei may be important areas for this type of sensory integration (McDonald, 1992).

Figure 5. Schematic diagram depicting the intrinsic connections of the amygdaloid sub-nuclei. For schematic simplification, the anterior cortical nucleus has been illustrated together with the periamygdaloid nucleus and the posterior cortical nucleus with the amygdalohippocampal area. In this figure, the deeper nuclei are located on the top whereas the more superficial ones are at the bottom. This figure is adapted from McDonald (1992), p. 89.



Consistent across all of the mammalian species studied to date, an important characteristic of intraamygdaloid connections is that there are scarce projections from the medial, cortical, or central nuclei, or from the amygdalo-hippocampal area back to the lateral and basal nuclei. Consequently, the stream of information within the amygdala appears to be essentially unidirectional, following a lateral to medial trajectory (Price, Russchen, and Amaral, 1987).

A. The lateral nucleus

The lateral nucleus sends prominent efferents to the accessory basal nucleus and periamygdaloid cortex, an anatomical feature found not only in the rat, but also in the cat and monkey (Aggleton, 1985; Krettek and Price, 1978 b; Ottersen, 1982; Russchen, 1982 b). Additionally, this nucleus projects to the central, medial and posterior cortical nuclei, albeit less heavily. In the rat brain, no afferent interamygdaloid connections to the lateral nucleus have been identified (Price, Russchen, and Amaral, 1987).

B. The basal nucleus

The most anterior (magnocellular) division of the basal nucleus appears to share bidirectional connections with the most posterior (parvicellular) one. Both segments send dense efferents to layer II of the nucleus of the lateral olfactory tract, and to a lesser extent, to the medial division of the central nucleus (Krettek and Price, 1978 b, Ottersen, 1982). Additionally, the two divisions of the basal nucleus send projections to the medial and accessory basal nuclei and possibly to the amygdalo-hippocampal area (Price, Russchen, and Amaral, 1987).

C. The accessory basal nucleus

Because the accessory basal nucleus is difficult to identify in the rat brain, not much is known about its intrinsic connections. Thus far, only a projection to the central nucleus has been firmly established (Ottersen, 1982).

D. The medial nucleus

This nucleus appears to participate very poorly in the intrinsic intraamygdaloid circuitry, especially in the case of the rat, where only efferents have been identified, projecting to the posterior cortical nucleus (Nitecka, Amerski, and Narkiewicz, 1981).

E. The anterior cortical nucleus

According to Krettek and Price (1978 b), a certain degree of caution is necessary when interpreting anatomical data aimed at identifying intra-amygdaloid connections originating in the anterior cortical nucleus, since all investigations using injections into this area also have some involvement of the basomedial nucleus and the periamygdaloid cortex. However, when targeting this nucleus with only minimal involvement of its neighbouring structures, labelled material is found in various olfactory cortical areas, namely the prepiriform cortex, the periamygdaloid cortex, and the nucleus of the lateral olfactory tract (Luskin and Price, 1983), and these connections appear to be bidirectional (Krettek and Price, 1977 a,b,c; 1978 b). The anterior cortical nucleus also sends some efferents to the central and medial nuclei (Price, Russchen, and Amaral, 1987).

F. The periamygdaloid cortex

The olfactory bulb sends prominent efferents to the periamygdaloid cortex, which in turn shares profuse interconnections with other parts of the primary olfactory cortex (Lushkin and Price, 1983). Other projections of the periamygdaloid cortex have been identified to the lateral, basal, central, and medial amygdaloid nuclei (Ottersen, 1982; Wakefield, 1980).

G. The posterior cortical nucleus and amygdalo-hippocampal area

Traditionally, it has been somewhat of a challenge to distinguish between the projections of the posterior cortical nucleus and the amygdalo-hippocampal area, since these two structures are located very close to each other (Price, Russchen, and Amaral, 1987). Considering these two areas as a region, Ottersen (1982) has found that its major efferents terminate in the medial nucleus.

H. The central nucleus

Although the central nucleus is the amygdaloid area which receives the greater majority of intrinsic connections, it sends scarce efferents to other areas of the amygdaloid complex (Price, Russchen, and Amaral, 1987). Two regions that appear to receive such projections are the medial and anterior cortical nucleus, although this has not been firmly established (Price and Amaral, 1981, Price, Russchen, and Amaral, 1987).

I. Interhemispheric intraamygdaloid connections

Interhemispheric communication between the amygdaloid complexes are achieved through the anterior commissure. The posterior cortical nucleus appears to project homotopically to the other hemisphere (Nitecka, Amerski, and Narkiewicz, 1981), and it also projects to the contralateral medial nucleus (Ottersen, 1982), whereas the basal and accessory basal nuclei porject to the contralateral central, medial, and lateral nuclei (McDonald, 1992). A modest homotopic commissural projection has also been identified in the nucleus of the lateral olfactory tract (Luskin and Price, 1983). Before crossing in the anterior commissure, these projections course through the ipsilateral stria terminalis (Price, Russchen, and Amaral, 1987). Many of these fibers appear to make synaptic connections in the bed nuclei of the anterior commissure and contralateral stria terminalis, suggesting the existence of secondary projections to other contralateral structures such as the hypothalamus (Dauth, Dafny, and Gilman, 1976). The results of electrophysiological studies also support the existence of amygdaloid interhemispheric connections in the cat (Gloor, 1955) and rat (Dauth, Dafny, and Gilman, 1976) (see section 2.3.2.6 of this introduction).

2.3 FUNCTIONAL ASPECTS OF THE AMYGDALOID COMPLEX

"[...] the amygdala is an important nodal point in the adaptive neural response to an environment with constantly changing affective contingencies. For this reason, the amygdala undoubtedly plays an influential role in a wide range of behaviors." Amaral et al. (1992) p. 57.

2.3.1 Overview

As emphasized in the preceding sections of this document, the amygdala is an anatomically heterogeneous structure that deserves to be considered more as a complex than as a single nuclear entity. Given its complexity, it is not surprising that this region has been implicated in a wide variety of behaviours and pathologies, ranging from the control of heart rate, feeding, sexual behaviour, and the mediation of sensory associative learning, to the various mental and emotional deficits associated with schizophrenia, senile dementia, and even autism. Although this thesis concerns itself primarily with the modulatory influence of the amygdaloid complex on MFB reward pathways, and thus, emphasis in this document will be placed on such factors as motivation and reward, other functional aspects of this structure cannot be ignored and deserve at the very least a brief overview.

Earlier, it was pointed out that the apparent heterogeneity in the amygdaloid complex appears to be moderated by the numerous afferent and efferent connections of its subdivisions and their considerable intraamygdaloid links. Accordingly, the various nuclei, although anatomically discrete, probably work collectively to modulate a variety of behaviours. Similarly, while the number of roles played by the amygdaloid complex initially seems astonishing, there is

in fact an obvious line of continuity in much of the literature on amygdaloid function.

The most general interpretation of the function of the amygdaloid complex appears to be related to providing "emotional substance" to sensory stimuli (Price, Russchen, and Amaral, 1987). For instance, in the much-documented "Kluver-Bucy syndrome" (see following section of this document), primates that have undergone bilateral lesions to large areas of the amygdaloid complex no longer show fearful behaviour towards humans and are unable to discriminate food from non-edible objects (see Fonberg, 1972) . Such inadequate behaviours are thought to stem from the animal`s incapacity to exhibit the appropriate emotional response to the sensory experience with which it is confronted (Price, Russchen, and Amaral, 1987).

These and other behavioural effects of amygdaloid damage have led to the conjecture that the primary role of the amygdaloid complex is to provide species-specific interpretations to daily experience with relation to survival-oriented preferences or previous experience, and to consequently organize the appropriate hormonal, autonomic, and behavioural responses (Davidson et al., 1999; Fonberg, 1972; LeDoux, 2000). In this context, one can easily understand that amygdaloid dysfunction would influence a vast range of activities, from the singling out of suitable food or mates, to performing the appropriate behaviours in social situations. This idea is also consistent with the notion that amygdaloid damage may disrupt associative learning, resulting in a misinterpretation in the magnitude and extent of the importance attributed to a given reinforcement (Price, Russchen, and Amaral, 1987). This topic, the potential influences of the amygdaloid complex in reward, is most relevant to this thesis and will be further elaborated in subsequent sections. In the following paragraphs, some of the other behaviours and pathologies which have been linked to amygdaloid function and may contribute to the understanding of the

modulator influences of this incredibly complex region will be briefly enumerated.

A large body of data suggests the existence of cross-talk between the amygdaloid complex and the hypothalamus in the control of eating and drinking behaviours (Fonberg, 1969; Fonberg and Delgado, 1961; Gallo, Roldan, and Bures, 1992; King et al., 1994, 1996 a and b; Montgomery and Singer, 1975; Morgane and Kosman, 1960; Nishijo et al., 2000; Saad et al.,1994). For instance, lesions of the basolateral amygdaloid nucleus have been shown to result in hyperphagia (Morganeandand Kosman, 1960), while its electrical stimulation inhibits feeding behaviour (Fonberg and Delgado, 1961). In most cases, the results of these investigations have been interpreted as evidence for amygdaloid modulation of autonomic hypothalamic function.

These early findings appear to still hold true today. Nishijo et al (2000), for instance, recorded neuronal activity in the amygdala and hypothalamus of rats during the ingestion of different tasting solutions as well as during discrimination tasks of conditioned auditory stimuli preceding the presentation of these solutions. They found that the activity of both hypothalamic and amygdaloid neurons was changed when animals had to regulate ingestive behaviour by learning a new stimulus associated with food and by being exposed to stress. These findings were interpreted as evidence that the hypothalamus works in concert with the amygdala to influence the regulation of ingestive behaviour.

The involvement of the amygdaloid complex in emotion, motivation, learning and memory has been known for many years (see Aggleton, 1992; McGaugh, Cahill, and Roozendaal, 1996; Packard and Teather, 1998). Indeed, investigators have devoted entire careers to the elaboration of models aimed at understanding the contribution of the amygdaloid complex in memory encoding, storage, and retrieval, using such paradigms as conditioned fear (Davis, 1992;

Shi and Davis, 1999; Wilensky, Schafe, and LeDoux, 2000) and conditioned arousal and sensory information processing (Kapp et al., 1992) models. As a result of these strategies, it has been firmly established that the central nucleus of the amygdala is a decisive component of a forebrain system for associative learning (Gallagher and Holland, 1992).

Furthermore, the last decades have witnessed an extraordinary escalation in research linking the amygdaloid complex with a panoply of pathologies such as amnesia (McGaugh et al., 1992), senile dementia (Mann, 1992), epilepsy (Adamec, 1998, 2001; Adamec and Shallow, 2000; Adamec and Young, 2000; Gloor, 1992; Helfer, et al., 1996), schizophrenia (Reynolds, 1992), and even autism (Baron-Cohen et al., 2000). As early as the 1950's, studies performed on human subjects demonstrated abnormal electrical activity from limbic structures of schizophrenic patients. Disorders of the temporal lobe, such as temporal lobe epilepsy, are commonly associated with schizophrenic-like symptoms to such an extent that this form of epilepsy is considered by some investigators to be a predisposition of schizophrenia (Reynolds, 1992). Given the tendency of the amygdaloid complex to exhibit seizure-like activity in response to damage and/or activation, amygdala kindling has profusely been used as a model to study brain plasticity and epilepsy (Cain, 1992). Because there appears to be an inverse relationship between the incidence of seizures and psychosis as well as a correlation between the presence of seizures and depression, some researchers have speculated whether a kindling-like effect in the human brain could underlie the development of human epileptic states and the neural changes leading to the emergence of behavioural disorders (Cain, 1992).

Autism has been defined as "a neuropsychiatric condition that disrupts the development of social intelligence" (Baron-Cohen et al., 2000, p. 355). As earlier illustrated using the

example of the Kluver-Bucy syndrome, the amygdaloid complex has long been suspected of playing a major role in social behaviour, to the point that recently amygdaloid abnormality has been speculated to be one possible cause of autism (Baron-Cohen et al., 2000). It would appear from data based on functional magnetic resonance imaging that the amygdala is not activated during tasks of inference in patients with autism, in contrast to normal human subjects. Furthermore, post-mortem studies of individuals diagnosed as autistic reveal microscopic pathology associated with abnormal growth in the amygdaloid complex (Bauman and Kemper, 1994; Rapin and Katzman, 1998). These observations suggest that the amygdaloid complex is involved in the development of what has been called "social intelligence", as defined by Brothers (1990).

Alzheimer's disease and dementia, two memory pathologies ascribed to ageing, have also been associated with amygdaloid malfunction. Early descriptions of the amygdaloid complex in an 82-year old woman suffering from Alzheimer's disease date back to 1928 (Grunthal, 1928, in Mann, 1992). In this report, neuronal loss, reactive astrocystosis, and senile plaques were observed to be extensively present, especially at the level of the amygdaloid cortical nuclei. More recently, marked reductions in the size of the amygdaloid complex have been reported in most cases of Alzheimer's disease (Brady and Mufson, 1990). An overall atrophy of the amygdala that ranges from 14 to 70% of control values , presumably reflecting neurodegenerative processe in this part of the brain, additionally appears to be a feature of dementing disorders such as Pick's disease, Huntington's chorea, and Steele-Richardson syndrome (see Tomlinson and Corsellis, 1984).

2.3.2 The amygdaloid complex in motivation and reward

2.3.2.1 First insights into the role of the amygdala in emotion and motivation: The Kluver-Bucy syndrome

Since its infancy, one of the dominant preoccupations in psychology has been to understand emotion and motivation. Today, one of the greatest challenges in the fields of neuroscience is to uncover the neural mechanisms underlying these states, and one of the most exciting contemporary advancements in the conceptualization of amygdaloid function is its probable role in various reward-related processes. Historically, the importance of the amygdala to emotional and motivational processes was not well recognized until it was ascertained that the striking emotional changes characterizing the Kluver-Bucy syndrome were attributable to amygdaloid damage (LeDoux, 1992).

Already more than a century ago, Brown and Schafer (1888, in LeDoux, 1992), followed by Kluver and Bucy (1937), discovered that extensive lesions of the temporal lobe changed previously wild and aggressive monkeys into tame, indifferent animals. Prior to surgery, the animals would attack any human attempting to handle them. Following the lesion, however, the creatures could be touched and teased without consequence. These and subsequent studies of the Kluver-Bucy syndrome have substantially contributed to the view that the amygdaloid complex is a fundamental structure in the processing of emotion. Specifically, this work has led to the conception that emotional and motivational significance are given to sensory stimuli following transmission of sensory input to the amygdala. When a large portion of the amygdaloid complex is lesioned, primates become tame towards investigators because the sight of a human is no longer coded as a threatening stimulus. Thus, it is believed that although stimuli are still

perceived as objects when the amygdala is damaged, they are no longer interpreted as emotionally significant objects (LeDoux, 1992).

2.3.2.2 Stimulus-reward association learning

In the past decades, investigators have designed tasks to directly test the hypothesis suggested by the Kluver-Bucy syndrome - that is, that the amygdaloid complex processes the affective significance of visual stimuli - by determining whether an animal is able to associate sensory stimuli with rewards. For instance, a classical paradigm used by Jones and Mishkin (1972) involved the substituting of the reward value of two stimuli. Animals were first taught to obtain a peanut located under stimulus A but not B. Once this task was reliably executed, the conditioning stimuli were switched so that the peanut was now located under stimulus B. While all subjects learned the initial task, animals with amygdala damage had great difficulty performing when the association between stimulus and reward was reversed while previous associations were unimpaired, suggesting that the amygdaloid complex is implicated in the modification of the association between visual stimuli and rewards.

2.3.2.3 The memory of reward

In light of the now-familiar Kluver-Bucy syndrome and other related observations, it may seem indisputable that the amygdala plays a crucial role in reinforcement, as defined by the process by which rewards and punishments influence what animals learn to do. Indeed, it is difficult to conceive the means by which an animal could acquire reinforced learning abilities if indifferent to rewards and punishments. However, the simple view that the amygdaloid complex

is involved in all forms of stimulus-reward association learning does not appear to be substantiated. Recently, one investigator suggested that the fundamental function of the amygdala is in providing specific associations between stimuli and reward (Gaffan, 1992). For example, rats will pay little interest to a novel but appealing food until tasting it. Thus, as suggested by Gaffan (1992), a deficiency of such emotional behaviours as previously mentioned following extensive damage to the amygdaloid complex could very well originate from a failure of associative memory towards the cost of that particular reward.

There is, in fact, ample evidence of abnormal associations between stimuli and the rewarding value of food following amygdaloid damage. In one such investigation (Brown, Latimer, and Winn, 1996), animals having received a bilateral intracerebral infusion of ibotenic acid into the sublenticular extended amygdala showed a tendency to work harder than controls to obtain sucrose pellets (indicating an increase in motivation towards the treat), as measured by their number of bar-presses. These results were interpreted as evidence that the sublenticular extended amygdala mediates the changing cost of a reward through mnemonic, rather than motivational, processes.

2.3.2.4 The concept of the "extended amygdala" and its relation to drug addiction and allostasis

In recent years, Koob (1998, 1999) has elaborated the idea according to which the specific neuronal circuitry of a region termed "the extended amygdala" is of decisive importance in understanding not only the mechanisms underlying drug reinforcement, but also, its relationship to natural reward systems. He has proposed that the extended amygdala reward system is a pivotal point for the emergence of "allostasis" in addiction. In contrast to "homeostasis", which

refers to stability within physiological systems, "allostasis" is defined as "a state of chronic deviation of the regulatory systems from their state of normal state of operation with establishment of a new set point" (Koob, 2001, p. 102). Thus, this mechanism implies that in a state of drug addiction, various physiological functions may be either activated or subdued, resulting in brain-organism interactive processes that ultimately prevail over local regulation. Because the allostatic model involves the whole brain, in an organism in a state of allostatis, factors such as experience, memories, anticipation and re-evaluation of cravings in expectation of physiological requirements are re-organized to perpetuate the pathology of addiction (Koob, 2001).

The allostatic state is believed to result from changes in the neural substrates of positive reinforcement, as well as from the recruitment of other reward and stress-related neuroadaptative systems. According to Koob (2001), the brain reward systems are altered with the development of addiction and of allostasis, for example by modifying the specific innervations of the mesolimbic dopamine system, which projects profusely to the extended amygdala (Koob, 1998, 1999).

Certain elements of the basal forebrain have been distinguished as plausible substrates for the positive rewarding effects of drugs and the negative reinforcement related to the allostatic state characteristic of addiction. Recent neuroanatomical and functional findings have brought forth an intriguing hypothesis according to which the "extended amygdala", a demarcated macrostructure within the basal forebrain, is formed by a common neural circuitry. (Gray, 1999; Koob, 1999). Originally coined by Johnston (1923), the extended amygdala area is composed of numerous basal forebrain structures: the BNST, the central medial amygdala, the medial portion

of the nucleus accumbens (mainly its shell portion) and the sublenticular substantia innominata (Koob, 1999). It borders the ventral striatum as it extends through the basal forebrain and associates with parts of the magnocellular basal forebrain (Gray, 1999).

The construct of the extended amygdala emerged from the fact that the amygdaloid complex is a system whose anatomical connections and neurochemical characteristics are much more similar to adjacent parts of the temporal lobe and basal forebrain rather than possessing its own unique features (Gray, 1999). With respect to linkage, for instance, the components of this region all receive afferent connections from limbic cortices, the hippocampus, basolateral amygdala, midbrain, and lateral hypothalamus. Additionally, all of the portions of the extended amygdala send efferents to the posterior medial (sublenticular) central pallidum, medial ventral tegmental area, numerous brain stem projections, and possibly most interesting from a functional point of view, a major projection to the lateral hypothalamus (Koob, 1999). This latter connection may suggest a significant functional link between the neurochemical and neurobiological basis of drug reinforcement with the long-theorized neurobiological substrates of BSR (Koob, 2001).

In the same vein, Gray (1999) has recently suggested that the ventral striatum, extended amygdala, and basolateral amygdala circuitry appear to play an important role in associating previously bland stimuli with the rewarding properties of a variety of reinforcing ones such as drugs, food, and sex. Although within this system, it has been suggested that the extended amygdala seems to be chiefly involved in the modulation of responses associated with rewarding stimuli only during the learning phase (Gray, 1999), others have found evidence implicating the extended amygdala in all sources of reinforcement (positive, negative, conditioned positive and

conditioned negative reinforcement) (Koob, 1999). Thus, it appears that the central extended amygdala, and more particularly its central part, plays a crucial role in sensory-reward neural associations.

2.3.2.5 Functional anatomy with relation to reward: Connections between the amygdaloid complex and the MFB

In the rat and other mammals, the amygdaloid complex is heavily interconnected to a multitude of other brain structures, including the olfactory bulb and primary olfactory cortex, the substantia innominata, the corpus striatum, the thalamus and hypothalamus, the brainstem, and the hippocampus (Price, Russchen, and Amaral, 1987). Here, we will only mention those fiber systems which are directly relevant to the studies presented in this thesis, thus focussing on the MFB and more specifically on the LH, on the BNST, and on the VTA.

Already more than half a century ago, investigators established that the fundamental circuitry for emotional and motivational behaviour, including its autonomic, somatic, and endocrine components, was situated in the hypothalamus and brainstem (for a review, see LeDoux, 2000). The amygdala appears to play a direct role in emotional and motivational behaviour by modulating the activity of these subcortical regions (McDonald, 1998). All of the amygdaloid sub-nuclei, with the exception of the lateral nucleus, project to the hypothalamus and to the BNST. Since a predominant proportion of the BNST efferents travel to the hypothalamus, this pathway potentially contributes an important di-synaptic trajectory for amygdaloid modulation on hypothalamic function (Price, Russchen, and Amaral, 1987).

Efferent fibers from most of the amygdaloid sub-nuclei leave the amygdala via two major

fiber systems: the stria terminalis and the ventrofugal bundle (ventral amygdalofugal pathway) (Isaacson, 1982). In the rat brain, the ventral amygdalofugal pathway comprises fibers which enter or exit the amygdaloid complex mainly at the rostral levels of its dorsomedial extremity. In contrast, the stria terminalis arises predominantly from the central and medial nuclei of the amygdala, and to a lesser extent, from the basolateral nucleus (Isaacson, 1982). At least three components of the stria terminalis-dorsal, ventral, and commissural- have been identified in the rat brain. De Olmos (1972) has described the dorsal component as sending fibers to the BNST, the basal portion of the lateral septal nucleus, the posterior-medial portion of the nucleus accumbens septi, the olfactory tubercule, parts of the anterior olfactory nucleus, the granular layer of the accessory bulb, the medial preoptic area, the portion located around the ventromedial nucleus of the hypothalamus, and an area located immediately before the mammillary nuclei.

The ventral component of the stria terminalis projects to the bed nucleus of the stria terminalis, the junction of the preotic area and the hypothalamus, the ventro-medial nucleus of the hypothalamus, and the premammillary area. Via the anterior commissure, the commissural and dorsal components of the stria terminalis connect the amygdalae of the two hemispheres (see section 2.2.3 I of this document) (Isaacson, 1982). At the termination of the stria terminalis and the ventral amygdalofugal pathways, a redundancy appears to take place, in that one component of the limbic system projects to another area over two or more different routes (Isaacson, 1982). Price and Amaral (1981) also share this view, pointing out that throughout their trajectories, fibers from one bundle appear to join fibers in the other one. For instance, fibers that initially course in the ventral amygdalofugal pathway pass through the internal capsule and link themselves to the stria terminalis, and fibers from both pathways end in the lateral preoptic area.

However, Isaacson (1982) suggests that this organization does not necessarily imply redundancy in the system, but rather, an indication that each of the amygdaloid sub-components has its unique pattern of projection in the hypothalamus. This characteristic is interpreted as suggesting the existence of different routes of regulatory control, and perhaps even dissimilar types of control by distinct cell groups of the amygdala (Isaacson, 1982).

A. Amygdaloid efferents to the bed nucleus of the stria terminalis and hypothalamus

The amygdaloid nuclei which are located at the most medial portion of the complex profusely project not only to the medial part of the BNST, but also more directly to the medial amygdaloid nucleus and the medial hypothalamus (Eiden et al., 1985; Kawata et al., 1985). The basal amygdaloid nucleus and the central nucleus contribute fibers both to the lateral portion of the BNST and to the lateral hypothalamus (Price, Russchen, and Amaral, 1987), whereas the accessory basal nucleus sends efferents only to the medial and lateral portions of the BNST (Krettek and Price, 1977 a, 1978 a).

At the second "relay" stage, the lateral division of the BNST projects to the lateral hypothalamic area, the ventral midbrain and central gray, the pontine parabrachial nucleus and the vagal nuclei and medullary reticular formation (Holstege, Meiners, and Tan, 1985), whereas the medial part of the BNST projects primarily to the medial hypothalamus (Turner and Knapp, 1976; Swanson and Cowan, 1979). In general, the fibers originating in the amygdaloid complex course either ventrocaudally from the BNST or medially from the ventral amygdalofugal pathway, terminating in the preoptic region and anterior hypothalamus (Price, Russchen, and Amaral, 1987). Most of those fibers commence in the medial and anterior cortical nuclei, at least

in the case of the rat (Price, Russchen, and Amaral, 1987).

The ventromedial nucleus of the hypothalamus is the recipient of a very salient projection from the amygdala, which divides itself into a portion terminating in a shell-like space surrounding the nucleus, and into another portion ending in the heart of the ventromedial hypothalamic nucleus (De Olmos, 1972). The latter fibers begin in the medial and accessory basal nuclei, and probably in the anterior cortical nucleus as well, whereas the projections ending in the shell-like space of the ventromedial nucleus of the hypothalamus originate in the amygdalo-hippocampal area (passing through the stria terminalis) and from the ventral portion of the subiculum (Price, Russchen, and Amaral, 1987).

In summary, the amygdaloid complex projects most abundantly to the lateral area of the hypothalamus, with most of its fibers originating in the central nucleus, and to a lesser extent, in the anterior cortical and medial nuclei (Price and Amaral, 1981).

B. Amygdaloid efferents from the hypothalamus to the amygdaloid complex

It has been clearly established that the hypothalamus sends heavy efferents to the amygdaloid complex, the bulk of which originates in the ventromedial nucleus, the lateral preoptic area and the lateral hypothalamus, and ends mainly in the medial division of the central nucleus, in the medial nucleus and in the parvicellular portions of the basal and accessory basal nuclei (Amaral, Veazey, and Cowan, 1983; Russchen, 1982 a). Other projections are thought to emanate from the arcuate, premammillary, and supramammillary nuclei, entering the amygdaloid complex trough the ventral amygdalofugal pathway. However, the end-point of these fibers has not yet been identified (Price, Russchen, and Amaral, 1987).

C. Connections between the amygdaloid complex and the ventral tegmental area

Although the most important amygdaloid projections to the MFB terminate in the hypothalamus, fibers from the ventral amygdalofugal pathway which project profusely to the MFB and past the perifornical LH terminate in the VTA (Hopkins and Holstege, 1978; Prewitt and Herman, 1998; Van der Kooy et al., 1984). Efferents from the A9 and A10 VTA cell groups include dense projections to the amygdaloid nuclei, mostly to the central and basolateral ones (Loughlin and Fallon, 1983, Oades and Halliday, 1987). In addition, 6-OHDA lesions applied to the VTA have been found to reduce dopamine levels in the amygdala by about 90%, indicating that a significant portion of VTA efferents to the amygdaloid complex are rich in dopamine (Oades and Halliday, 1987).

2.3.2.6 Electrophysiological studies of amygdaloid-MFB connectivity

Numerous electrophysiological studies conducted over the past five decades indicate that the amygdala shares functional links with the MFB. As early as the 1950's and 60's, investigators repeatedly showed that electrical stimulation in the amygdala affects the firing pattern of numerous hypothalamic neurons (Dreifuss, Murphy, and Gloor, 1968; Egger, 1967; Gloor, 1955; Stuart, Porter, and Adey, 1964; Tsubokawa and Sutin, 1963). In one such study (Gloor, 1955), single or continual electrical shocks applied to various subdivisions of the amygdaloid complex evoked neural responses in the septal nuclei, the proptic area, the hypothalamus, and the midbrain tegmentum. Interestingly, two types of response latencies were identified, now recognized as the two projection pathways linking the amygdala to the MFB (the stria terminalis and the ventral amygdalofugal pathway). Short latency responses were found in the stria terminalis following

corticomedial amygdaloid stimulation, whereas stimulation of the basolateral nucleus evoked either no or very long latency ones in the stria. The response latencies recorded in the anterior hypothalamus and preoptic area, which were mediated by the ventral amygdalofugal pathway, were shorter than those infrequent responses to basolateral stimulation recorded in the stria terminalis. These observations led the investigators (Gloor, 1955) to suggest that the main outflow from the basolateral amygdaloid nuclei to the hypothalamus does not appear to travel through the stria terminalis but is probably relayed through the corticomedial nuclei of the amygdaloid complex.

In a latter electrophysiological investigation, Gloor and his collaborators (1969) confirmed that the impulses originating in various subnuclei of the amygdaloid complex and mediated by the two efferent fiber systems elicit different responses in the hypothalamus. Moreover, they found that repetitive amygdaloid stimulation of the basolateral amygdaloid subnuclei produced an increase in the rate of firing in hypothalamic neurons, indicating that neurons in this portion of the amygdaloid complex have the ability to change the discharge pattern of hypothalamic neurons as a function of their own discharge rate (Gloor, Murphy, and Dreifuss, 1969).

Gloor and his group's (1969) most important contribution in this regard was to show that stimulation of the basolateral and corticomedial amygdala elicits two morphologically distinct neuronal discharge patterns in the ventromedial hypothalamus. Electrical stimulation of the basolateral amygdaloid nucleus gave rise to specific patterns of neuron discharge linked with characteristics of the neurons present in the ventral amygdalofugal pathway. In contrast, corticomedial stimulation elicited patterns of activity associated with the stria terminalis. Others

(Oomura, Ono, and Ooyama, 1970) have additionally provided evidence that the inhibitory effect of amygdala stimulation on LH neuronal discharge patterns is due to a long lasting inhibitory postsynaptic potential achieved through the stria terminalis.

More recently, Han and Ju (1990) demonstrated the existence of reciprocal connections between the oval nucleus of the BNST, the amygdaloid central nucleus, and the LH. In that investigation, spontaneous unit discharges in 53% of the neuronal population of the oval nucleus of the BNST, which could in turn be influenced by stimulation of the central nucleus of the amygdala, were modified following focal stimulation of the LH. Moreover, neurons in the oval nucleus of the BNST were antidromically activated by focal stimulation of either the central nucleus or the oval nucleus of the BNST.

In an investigation designed to study bilateral amygdaloid projections to the hypothalamus, Dauth and his collaborators (1976) recorded single unit activity from neurons in the anterior and ventromedial hypothalamus as well as in the mesencephalic reticular formation following ipsi and contralateral amygdaloid stimulation. In this study, the average firing rate of neurons in the ventromedial hypothalamus and mesencephalic reticular formation, but not in the anterior hypothalamus, was the same whether the stimulation was applied to the ipsi or to the contralateral amygdaloid complex. These data provide strong physiological evidence towards the existence of functional connectivity between the amygdala and hypothalamus, both contra and ipsilateral, as well as between the amygdala and the mesencephalic reticular formation.

2.3.2.7 Behavioural and lesion studies of amygdaloid-MFB connectivity

One important indication that the amygdaloid complex is implicated in the processing of rewarding stimuli is its ability to mediate BSR (Egger, 1967; Kane, Coulombe, and Miliaressis, 1991 a and b; Panagis et al., 1995; Szabo, Rozowska, and Kolta, 1972; Touzani and Velley, 1998; Vachon and Miliaressis, 1992). Although maximum rates of self-stimulation are typically low (varying from as little as 3 to 37 bar presses/minute in general), reliable thresholds can be obtained in many sub-sites of the complex. In one self-stimulation mapping study (Kane, Coulombe, and Miliaressis, 1991 a), for instance, about 40% of the 126 amygdaloid test sites were found to support BSR, including the anterior amygdaloid area as well as the medial and the basolateral nucleus. In this investigation, the lateral amygdaloid nucleus was not found to promote this behaviour. As in other studies (Panagis et al., 1995; Touzani and Velley, 1998; Vachon and Miliaressis, 1992), self-stimulation obtained from the amygdaloid complex was typically accompanied by epileptiform signs, at least partially explaining the low rates of self-stimulation obtained from this region. Additionally, rate-frequency functions were extremely stable and seizures occurred only exceptionally.

The literature concerning the relationship between the amygdala and the MFB with respect to BSR is quite scarce. In an early study (Szabo, Rozowska, and Kolta, 1972), the administration of pulse pairs comprising LH pulses of variable current and fixed amygdala ones (just above threshold) produced only small ameliorations in rate and threshold compared to trains of LH pulses alone. In a more recent investigation undertaken to explore the interactions between amygdaloid and hypothalamic BSR, Kane and his colleagues (1991 b) evaluated whether pairing trains of rewarding LH and amygdala stimulation altered the thresholds obtained from

stimulation at either site alone. Regardless of the order of pulse presentation, they found no significant summation of paired-pulse stimulation when the currents associated with the two sites were chosen to produce similar frequency thresholds.

The results of an earlier study, however, suggest that it may be crucial to consider the specificity of amygdaloid subregions in modulating reward derived from MFB activation. Jackson and Gardner (1974) found that stimulation of the basolateral amygdala had no impact on LH self-stimulation rates, whereas corticomedial amygdaloid nucleus stimulation inhibited them. Waraczynski, Ng Cheong Tom, and Shizgal, (1990) observed that threshold values for rewarding LH as well as VTA stimulation in nine rats remained close to baseline levels following electrolytic lesions of the amygdalofugal pathway, as well as of the medial and basolateral sub-nuclei amygdaloid complex.

Interestingly, Kaada (1972, in Price, Russchen, and Amaral, 1987) was among the first researchers to oppose his epoch's prevalent notion that there was no distinction between the nuclei of the amygdaloid complex with relation to function. He instead suggested that the individual anatomical subdivisions of this complex play distinct roles in numerous behavioural functions, including feeding, reproduction and flight/defense reactions (Price, Russchen, and Amaral, 1987). Although the sub-nuclei and cortices of the amygdaloid complex are linked by intrinsic connections and therefore not isolated from each other (see section 2.2.3 of this document), recent anatomical, physiological, and behavioural data support the concept of individualized amygdaloid divisions, each playing a particular modulatory role in various behaviours and cognitive processes.

In view of the anatomical and functional characteristics of the amygdaloid complex on the one hand, and of the large body of experimental evidence pointing towards the existence of a link between this locus and the MFB on the other (elaborated in this Introduction), the first investigation of this thesis was designed. It was of an exploratory nature and aimed at studying the functional relationship between the LH - a noteworthy BSR site - and the amygdaloid complex via the use of a metabolic marker, cytochrome oxidase. We reasoned that if there is in fact such a link, then lesioning the LH would result in an alteration of cytochrome oxidase activity in functionally related amygdaloid sub-sites.

EXPERIMENT 1

Published as: Miguelez, M., Bielajew, C., Diotte, M. and Shiao, R. (2001). Dynamic changes in cytochrome oxidase activity in the amygdala following lesions of rewarding sites in the lateral hypothalamus. **Behavioural Brain Research, 119**: 103-110.

1. Abstract

The aim of this study was to evaluate neural changes in oxidative metabolism in amygdaloid sub-nuclei following unilateral electrolytic lesions of lateral hypothalamic sites which supported brain stimulation reward (BSR). A histochemical analysis of cytochrome oxidase activity, comparing lesioned to non-lesioned sides in the amygdala, revealed a significant reduction of oxidative metabolism in the cortical nucleus and, to a lesser degree, in the adjacent piriform cortex; this effect was observed two to four weeks after the lesion, with complete recovery by the eighth week in the case of the cortical nucleus only. No particular pattern in cytochrome oxidase activity was detected in other amygdaloid sub-nuclei that were examined, including the basolateral and medial nucleus. The most pronounced decreases in metabolic activity were located at roughly the same level, corresponding to the posterolateral and posteromedial levels of the cortical nucleus and just anterior to the amygdalopiriform transition. These results suggest that within the amygdaloid complex, the cortical sub-nuclei and possibly the neighbouring piriform cortex contribute more to modulating lateral hypothalamic self-stimulation than components of the central extended amygdala.

2. Introduction

The existence of abundant anatomical connections between the amygdala and the hypothalamus has been well-documented. Afferents from the amygdala reach the hypothalamus via two major routes. The first of these connections, the stria terminalis, is a compact bundle of fibers arising predominantly from the medial, central, and basomedial nuclei of the amygdala which provides the main efferent projection from the amygdala to the hypothalamus (De Olmos,

1972; Hall, 1972; Han and Ju, 1990; Prewitt and Herman, 1998). The second link is the ventral amygdalofugal pathway; this group of fibers originates in the piriform cortex and basolateral nucleus, providing a diffuse network of efferent and afferent connections between the amygdala and the hypothalamus (De Olmos, 1972; Hall, 1972; Prewitt and Herman, 1998). Electrophysiological studies also support this view. For example, amygdaloid stimulation has been reported to induce assorted patterns of neuronal responses in different parts of the hypothalamus (Dauth, Dafny and Gilman, 1976; Dreifuss, Murphy and Gloor, 1968; Egger, 1972; Gloor, 1975; Gloor, Murphy and Dreifuss, 1969; Han and Ju, 1990; Oomura, Ono and Ooyama, 1970; Renaud, 1976; Renaud and Hopkins, 1977; Van Atta and Sutin, 1971).

An interesting functional significance of the amygdala appears to lie in its capacity to orchestrate the interplay of different structures that mediate a variety of behaviours. Several investigators have shown that lesions of the amygdala can impair an animal's learning and long-term mnemonic processes (Bianchin et al., 1999; Hatfield and McGaugh, 1999) in many behavioural paradigms, including conditioned taste-aversion (Reilly, 1999) and conditioned emotional responses (Shi and Davis, 1999). The amygdala also appears to modulate certain sensory modalities, such as those related to the recognition of pain and the processing of visual and auditory stimuli (Deboer et al., 1999), and has also been suggested to be a sensory-reward association area. Finally, animals with lesions of the amygdala are known to become very tame and submissive (Fonberg, 1973).

Both the hypothalamus and amygdala have long been considered to play an important role in the regulation of many emotional and motivational behaviours in mammals (Fonberg, 1969; Salinas, Packard and McGaugh, 1993). For instance, several reports have found evidence of an

interaction between these two structures in the control of drinking (Grossman, 1964; Singer and Montgomery, 1969) and feeding (Brown, Latmer and Winn, 1996; Fonberg, 1974; Fonberg, 1975; King et al., 1996; Morgane and Kosman, 1960; Nishijo et al., 2000; Pomonis et al., 1997). In all of these studies, the findings have been interpreted in terms of amygdaloid modulation of hypothalamic functioning.

The link between the hypothalamus and the amygdala has also been explored using the brain stimulation reward (BSR) paradigm. While it is clear that both the lateral hypothalamus (LH) (Bielajew, 1991; Nakahara et al., 1999; Panagis et al., 1995) and the amygdala (Dreifuss, 1972; Egger, 1967; Kane, Coulombe and Miliaressis, 1991; Panagis et al., 1995; Sokoloff, Kennedy and Smith, 1983; Szabo, Rozowska, and Kolta, 1972; Touzani and Velley, 1998; Vachon and Miliaressis, 1992) support self-stimulation, their interrelationship remains unclear. Current thresholds for BSR derived from the LH have been found to decrease following ipsilateral stimulation of the amygdala (Touzani and Velley, 1998). On the other hand, Kane et al. (1991) found that although the rewarding value of amygdaloid stimulation is comparable to that produced by hypothalamic stimulation, there appears to be a relative independence between these structures with respect to reward. No significant summation of the rewarding effects elicited by concurrent LH-amygdala stimulation was observed, contrary to the results typically obtained when the LH is paired with other medial forebrain bundle self-stimulation sites (Bielajew and Shizgal, 1980, 1986; Shizgal et al., 1980). In such cases, stimulation effectiveness has been shown to increase by as much as 80%. Still others have observed that stimulation of the basolateral portion of the amygdala had no effect on thresholds for rewarding LH stimulation while corticomedial amygdaloid nucleus stimulation inhibited it (Jackson and Gardner, 1974).

More recently, unilateral lesions of the LH produced by ibotenic acid have been shown to reduce rates for amygdaloid self-stimulation without altering current thresholds (Touzani and Velley, 1998). These data suggest that the rewarding effects of the central nucleus of the amygdala do not stem from activation of LH outputs; in fact, the reduction in rates is interpreted as resulting from lesion-induced increases in the frequency of seizures that occur following amygdaloid stimulation. Likewise, Warazcynski et al (1990) have demonstrated that electrolytic lesions which damage the principal amygdaloid sources of descending medial forebrain bundle fibers had no significant impact on the effectiveness of rewarding LH stimulation. In this regard, however, it should be noted that an absence of persistent decreases in reward appears to be a general feature of lesion studies, regardless of the location of the injury (Simmons, Ackermann and Gallistel, 1998; Waraczynski, Carlton and Perkins, 1998; Waraczynski, NgCheong Ton and Shizgal, 1990; Waraczynski, Perkins and Acheson, 1999).

Notwithstanding the above results, the role of the amygdala, particularly that part termed the "extended" amygdala (Johnston, 1923) has become a research focus in the area of drug reward (Koob, 1999). The structures that are believed to participate in producing and maintaining addiction are part of the same circuit that supports BSR in the basal forebrain. Hence, the present study was undertaken with the purpose of further exploring amygdala-LH interactions, using the endogenous metabolic marker, cytochrome oxidase (Wong-Riley, 1989).

A number of approaches have been used to study patterns of activity in neurons. Although the 2-deoxy-D-[^{14}C]glucose autoradiographic method has been commonly employed to map short-term metabolic activity during BSR (Porrino et al., 1984; see Sokoloff, Kennedy and Smith, 1983), histochemical analysis of cytochrome oxidase, a key enzyme in cellular

metabolism, has been shown to be an effective method of detecting long-term patterns of neuronal activity (Collier et al., 1997; Harley and Bielajew, 1992; Lopez et al., 1997; Nobrega et al., 1993; Wong-Riley, 1989). Cytochrome oxidase reaction product is distributed unequally throughout the neuron with the largest concentration in the dendritic region (Wong-Riley, 1989). Studies have demonstrated a correlation between areas of high functional activity and cytochrome oxidase levels (see Hevner, Liu and Wong-Riley, 1995).

Previous work in our laboratory has shown that generally, chronic BSR causes unilateral increases in cytochrome oxidase (Bielajew and Fouriezos, 1986) and that quantitative comparisons of stimulated-to-unstimulated sides of the LH reveal differences in cytochrome oxidase activity; the largest differences are found in mesocorticolimbic structures, suggesting that these may be involved in medial forebrain bundle self-stimulation (Bielajew, 1991). In this tradition we report the results of the present study which was an examination of the effects of lesions applied to rewarding LH sites on cytochrome oxidase activity in selected amygdaloid sub-nuclei.

3. Methods

3.1 Subjects and surgery

Nineteen male Long-Evans rats, weighing between 300 and 400 g at the time of the surgery, were anaesthetized with 65 mg/kg of sodium pentobarbital. Stainless steel monopolar electrodes were bilaterally implanted to target LH areas. The stereotaxic coordinates, based on the atlas of Paxinos and Watson (1986), were 3.0 mm posterior to bregma, 1.6 mm lateral to the midsagittal suture, and 8.3 mm below the skull surface measured at bregma. Following surgery,

rats were allowed a post-recovery period of one week. Throughout the experiment, they were housed in separate cages and kept on a 12-h light/dark cycle with light onset at 0700 h. Purina rat chow and water were freely available; body weight and food consumption were monitored regularly.

3.2 Screening and stabilization

Following recovery from surgery, the animals were screened for self-stimulation in a standard test cage using conventional shaping procedures. Stimulation parameters consisted of 0.5 s trains of monophasic cathodal rectangular pulses of 0.1 ms duration with the current set at approximately 300 μA and the frequency at 40 Hz. Sixteen subjects showed vigorous responding with minimal motoric side-effects. Rats were randomly assigned to one of four groups (four rats per group) according to a schedule of cytochrome oxidase analysis of either 1, 2, 4, or 8 weeks post-lesion. For each animal, rate-frequency functions were collected daily in order to establish a frequency threshold value. These functions were obtained by conducting a succession of 60 s trials, during which the current was held constant and the frequency of pulses was decreased in 0.1 $\log_{10}$ steps each trial from a value that produced maximum bar-pressing to one that generated few or no responses. The thresholds were interpolated from the rate-frequency function and corresponded to the value associated with a half-maximum rate; the functions were collected daily until the thresholds did not vary more than 0.05 $\log_{10}$ units for three consecutive days.

3.3 Lesions and cytochrome oxidase histochemistry

Once thresholds were stable according to the above criterion, rats were briefly anaesthetised with Halothane and electrolytic lesions were induced via the stimulating electrode by anodal current applied for 20 s at 6.0 volts. At 1, 2, 4, or 8 weeks following this procedure, each subject was perfused intracardially with an initial saline wash followed by a solution of 4% paraformaldehyde and 4% sucrose in a 0.1 M phosphate buffer. The brains were subsequently prepared for cytochrome oxidase histochemistry according to the protocol described by Wong-Riley (1979) with the exception that the reaction was carried out on slide-mounted sections rather than on free-floating tissue. Tissue sections were incubated in 160ml of 0.1 M phosphate buffer containing 54 mg cytochrome C (Sigma-Aldritch Canada), 88.8 mg diaminobenzidine (Sigma-Aldritch Canada) and 7.2 mg sucrose.

A microcomputerized imaging analysis system (Imaging Research Inc.) was used to obtain relative optical densities (rod) from amygdala subnuclei. Each reading, which was based on an outline of the entire structure, yielded a value represented along a continuous range of gray shades divided into 256 gray levels that were converted to a rod value by comparison to a clear portion of that slide. Hence, a rod value is defined as the log value of the ratio between the density of the structure and the clear part of the slide. Amygdala sections at four rostrocaudal levels corresponding to 1) the bifurcation point of the optic chiasm, 2) the posterior position of the dorsomedial amygdala nucleus, 3) the division of the ventromedial hypothalamus into central, dorsomedial, and ventrolateral nuclei, and 4) the incorporation of the posteroventral portion of the medial amygdala into the posterodorsal medial amygdala were chosen for analysis; these are equivalent to Paxinos and Watson (1986) atlas coordinates 1.8, 2.56, 3.14, and 3.60 mm behind

bregma.

4. Results

Sixteen animals displayed reliable self-stimulation behaviour. Their electrodes were histologically confirmed to the LH. A typical lesion from each group of subjects is shown in Figure 6; note that there was little difference in the lesion size across groups.

Figure 7 shows the average frequency threshold and maximum rate associated with each group. Kruskal-Wallis non-parametric tests (Howell, 1997) were used to assess variations in the average and maximum rates across groups; no significant difference was found. In addition, each animal's total charge was calculated using the formula Q = Ind where Q = the charge in microcoulombs (μC), I = the current in μA (300 μA), N = the number of pulses in the 500 ms stimulation train, and d = the pulse duration in sec (0.0001) (Gallistel, 1978). Little variation in total charge was observed, with mean values ranging from 0.70 to 0.75 μC across groups.

The rod differences between the lesion and the contralateral side were used as an index of the change in cytochrome oxidase activity induced by the lesion. An example of side-to-side differences in cytochrome oxidase staining is shown in Figure 8. There is obvious reduction in reaction product on the lesioned side in the cortical amygdaloid nuclei and adjacent piriform cortex, best evident at four (A - top plate) and eight weeks (B - bottom plate) post-lesion.

Figure 9 depicted the average group difference in rod for each of the analysed amygdala sub- structures, that is, the basolateral nuclei, the cortical nuclei, the medial nuclei, and the adjacent piriform cortex. In this figure, negative values indicate a greater enzymatic activity, whereas positive ones denote the reverse. Overall, a discrete reduction of oxidative metabolism

(increase in rod value) was observed, predominantly in the cortical nucleus area of the amygdala and to a lesser extent in the piriform cortex, when compared with the contralateral side. This effect was pronounced two to four weeks following the lesion, with some recovery apparent by the eighth week post-lesion. A non-parametric one way analysis of variance for independent samples (Howell, 1997), Kruskal-Wallis, revealed a significant difference in cytochrome oxidase activity over time following the LH lesion ($p < 0.0003$). As a post-hoc analysis, we applied the non-parametric Wilcoxon test (Howell, 1997) which showed that the cortical nucleus and adjacent piriform cortex contributed to the omnibus H. These differences occurred between weeks 1 and 4 in both cases and additionally between weeks 4 and 8 for the piriform cortex ($p \leq 0.05$). Note however that the obtained values for the results of the piriform cortex just met significance. These data are illustrated in Figure 10. The levels that best correspond to this pattern on the basis of visual inspection are the same for both structures (-2.56 according to the Paxinos and Watson (1986) atlas, that is, the posterolateral cortical amygdala nucleus (panel A) and that portion of the piriform cortex just before its transition point (panel B).

Figure 6. Tracings from the Paxinos and Watson atlas (1986) that best depict a typical lesion from each group of subjects identified on the basis of post-lesion period - either 1, 2, 4, or 8 weeks.

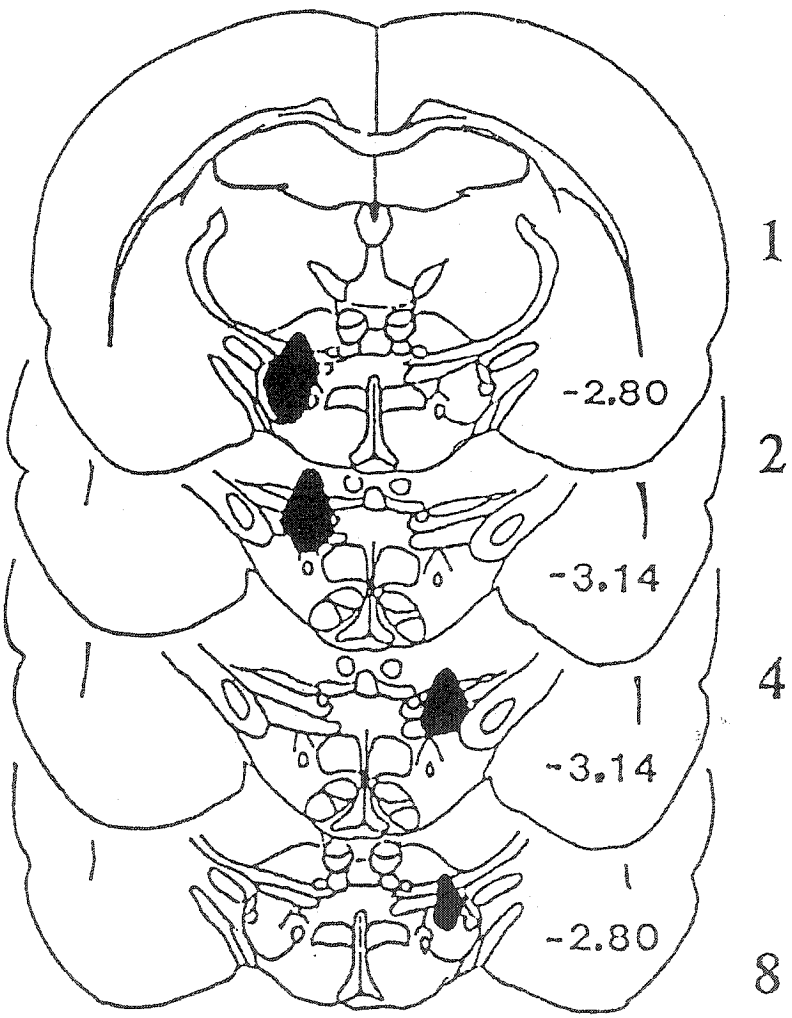


Figure 7. A. Average pre-lesion maximum rates and corresponding standard error of the mean across animals as a function of the post-lesion period - either 1, 2, 4, or 8 weeks. Each mean represents the data from four animals. **B.** Average frequency threshold value and corresponding standard error the mean across animals as a function of the post-lesion period - either 1, 2, 4, or 8 weeks. Each mean represents the data from four animals.

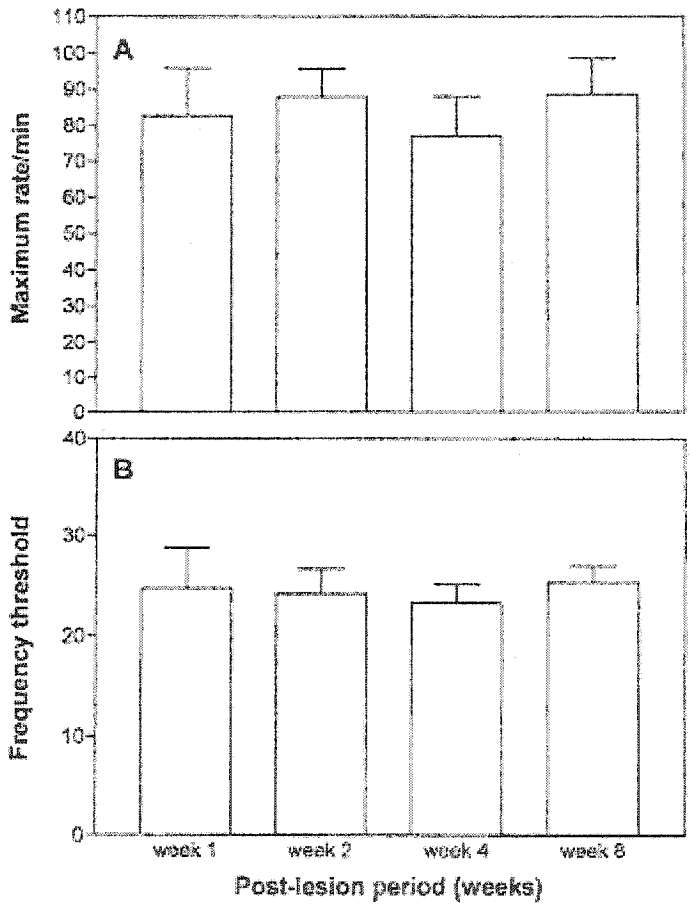


Figure 8. Sections that best illustrate side-to-side differences in cytochrome oxidase staining in cortical amygdaloid nuclei and piriform cortex at different times post-lesion with A (top section) representing four weeks and B (bottom section), eight weeks.

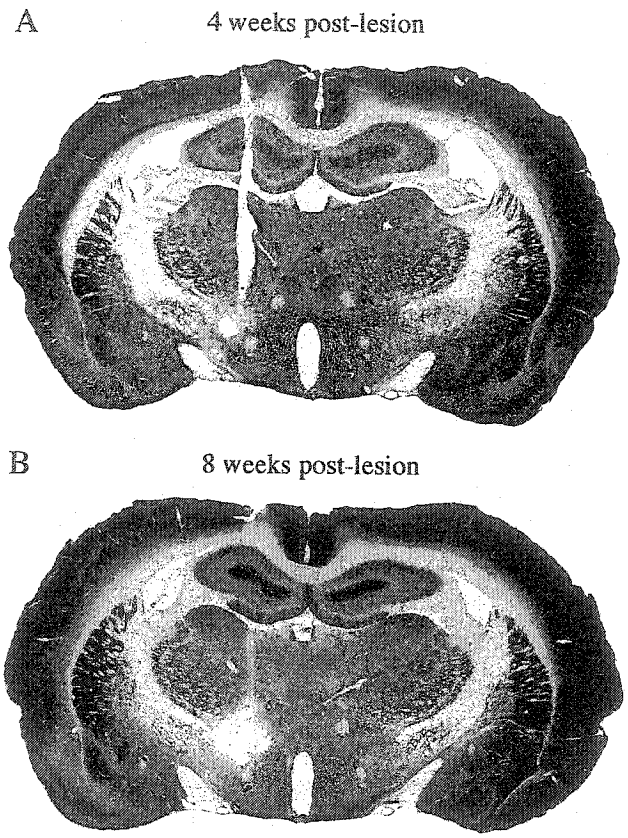


Figure 9. Results from a quantitative analysis of side-to-side differences in relative optical density (rod) in three amygdaloid structures, BN - Basolateral Nucleus, CN - Cortical nucleus, MN - Medial Nucleus, and PC - Piriform cortex, in the vicinity of the amygdala. Positive values indicate an overall decrease in enzymatic activity on the stimulated side and negative values the reverse. The group average is shown for each of the target sites as a function of post-lesion period.

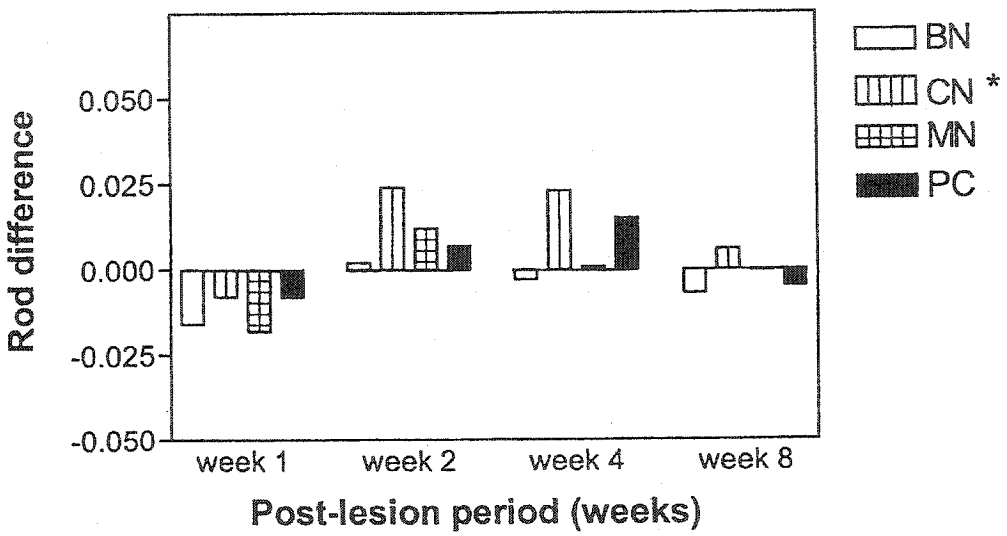
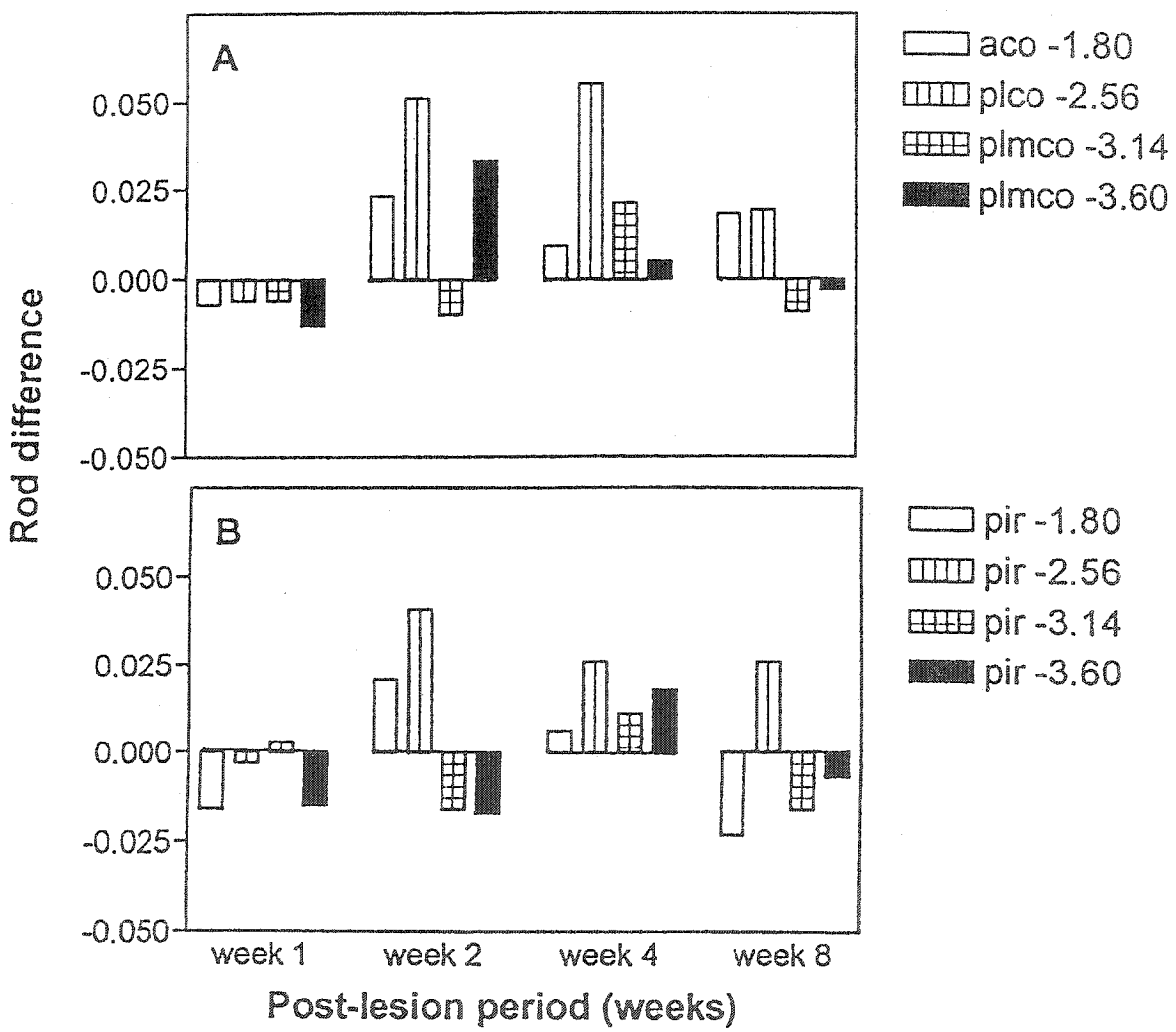


Figure 10 A. Results from a quantitative analysis of side-to-side differences in rod in the four cortical sub-nuclei of the amygdala: aco - anterior cortical amygdala nucleus, plco - posterolateral cortical amygdala nucleus, plmco - lateral and medial portions of the posteromedial cortical amygdaloid nucleus; these correspond to coordinates 1.80, 2.56, 3.14, and 3.60 posterior to bregma according to the Paxinos and Watson (1986) atlas. **B.** Rod results in the four sub-regions of the piriform cortex (pir) that were examined, corresponding to coordinates 1.80, 2.56, 3.14, and 3.60 posterior to bregma according to the Paxinos and Watson (1986) atlas.



5. Discussion

The results of this study show that electrolytic lesions of rewarding LH sites reduce oxidative metabolism of specific amygdaloid regions - in this case, the cortical nucleus, and to a lesser degree, the piriform cortex. Here, no significant changes were apparent in the basolateral and medial nuclei of the amygdala. Consistent with other studies in which cytochrome oxidase has been evaluated following neuronal ablation or inactivation (Wong-Riley, 1989), we observed discrete metabolic decreases most clearly two to four weeks following the lesion. Note differences in the four and eight week staining patterns shown in Figure 3, with less obvious accumulation of reaction product (left side) at four weeks than at eight.

The interpretation of this pattern is derived from the following considerations. Gradual adjustments in the activity and amount of the enzyme following an alteration of neuronal activity requires its synthesis, degradation and/or modification. Thus, a minimum amount of time of aoubt 2 weeks is required in order to achieve a new stable level of enzyme that is resolvable histochemically (Wong-Riley, 1989). In other words, since cytochrome oxidase histochemistry is based on the principle that the utilization of energy in neural tissue is determined by the overall functional activity of the neurons in question (DiRocco, Kageyama and Wong-Riley, 1989; Erecinska and Silver, 1989), this procedure reflects long-term metabolic activity rather than activity concerned with more immediate demands, such as in the case of 2-deoxyglucose (2-DG) autoradiography (Sokoloff, Kennedy and Smith, 1983). For this reason, significant cytochrome oxidase changes following manipulations that alter neuronal activity are usually not detectable for several weeks (Wong-Riley, 1989). On these grounds, we chose to conduct our analyses starting with the first week post-lesion.

Note that our results were likely not influenced by biases such as group number or behavioural particularities. Animals were randomly assigned to their respective groups (corresponding to the time at which the post-lesion analysis of cytochrome oxidase activity was performed), stimulation parameters were identical across groups with comparable frequency thresholds, total charge values, and maximum rates. Likewise, lesion size was not markedly different between subjects; however, the efficacy of the lesions in eliminating BSR was not determined. Finally, all participants in this study were free of any noticeable motor artifacts induced by the stimulation.

The cytochrome oxidase histochemical procedure was originally designed with the purpose of measuring long-term alterations of metabolic activity in mature neurons in response to some form of sensory deprivation (see Wong-Riley, 1989). Much of the initial work in this area has focussed on the visual system, showing progressive enzymatic reductions as a function of the nature, severity, and length of visual deprivation (Hendry and Jones, 1986; Horton and Hubel, 1981; Wong-Riley, 1984). In contrast, those interested in the circuitry underlying rewarding brain stimulation have used a variety of metabolic markers to measure cerebral activity following activation of rewarding brain sites (Arvanitogiannis, Waraczynski, and Shizgal, 1996; Bielajew and Fouriezos, 1986; Esposito et al., 1985; Gallistel et al., 1985; Hunt and McGregor, 1998; Konkle et al., 1999; Nakahara et al., 1999; Porrino et al., 1984, 1990; Sandner, Bielajew and Fouriezos, 1996). Although the analysis of amygdaloid cytochrome oxidase activity in response to rewarding BSR has not, to our knowledge, been specifically examined in other studies, no striking differences in amygdaloid enzymatic levels have been reported (Bielajew and Fouriezos, 1986). In the normal rat brain, investigators have shown that amygdaloid structures generally

display a moderate level of activity as compared to other subcortical regions (Harley and Bielajew, 1992; Hevner, Liu and Wong-Riley, 1995).

In contrast to cytochrome oxidase histochemistry, the use of short-term markers such as 2-DG to track metabolic changes in amygdaloid structures affected by BSR has yielded equivocal results. For example, significant unilateral increases of glucose utilization in both the central and basolateral amygdala have been reported as a result of rewarding ventral tegmental area stimulation (Porrino et al., 1984), while LH self-stimulation resulted in marked activity within the central amygdala (Porrino et al., 1990). Others have observed increases of 2-DG exclusively in the contralateral basolateral and central nuclei of the amygdala following ventral tegmental area self-stimulation (Esposito et al., 1984). Finally, there have also been reports of no changes in amygdaloid metabolic activity in response to rewarding brain stimulation (Gallistel et al., 1985). The use of c-fos, another marker frequently employed to evaluate brain regions activated by BSR, has provided evidence that the central amygdala is among those structures showing the highest immunoreactivity as a consequence of rewarding LH stimulation on the ipsilateral side (Hunt and McGregor, 1998). More moderate but significant increases in c-fos activity have also been reported by Arvanitogiannis, Waraczynski, and Shizgal (1996) in the bed nucleus of the stria terminalis, and by Nakahara et al (1999) in the same structure as well as in the general area of the amygdala. In the latter case, however, these effects were only observed following short-term exposure to BSR.

The measurement of cytochrome oxidase activity is considered to be a powerful tool for investigating structure/function relationships (Hevner, Liu and Wong-Riley, 1995; see Wong-Riley, 1989). In our case, the changes in amygdaloid enzymatic activity following lesions of

rewarding sites in the LH suggest that the cortical nucleus of the amygdala and possibly the piriform cortex are involved in the modulation of BSR, at least at the level of the LH. This interpretation is consistent with anatomical findings showing that although the amygdaloid nuclei seem to be a group of distinctive substructures (Cassell, Freedman and Shi, 1999), they all have abundant anatomical connections with the hypothalamus. The medial, central, and basomedial nuclei of the amygdala are at the origin of the stria terminalis, which is the dominant efferent projection linking the amygdala to the hypothalamus (De Olmos, 1972; Hall, 1972; Prewitt and Herman, 1998), and the piriform cortex as well as the basolateral nucleus of the amygdala provide a diffuse network of efferent and afferent connections between these two structures (De Olmos, 1972; Hall, 1972; Prewitt and Herman, 1998).

Because we do not know the relationship between the size of the effective stimulation field and that of the lesion, nor as mentioned earlier how effective our lesions were in reducing or eliminating BSR, we must consider the possibility that our results reflect damage to diencephalic input to cortical sub-nuclei of the amygdala, and are only accidentally related to BSR. A design in which a stimulation control group is incorporated (Gallistel et al., 1985; Porrino et al., 1984) would be helpful in addressing this question.

In addition to metabolic changes observed in cortical amygdaloid nuclei, smaller but longer-lasting decreases in enzymatic activity were found in the adjacent piriform cortex. There are at least two obvious interpretations of this result. The first is based on the role of the piriform cortex in the induction of limbic seizures (Nobrega et al., 1993). Several treatments of electroconvulsive shock-induced seizures result in significant increases in cytochrome oxidase activity in predominantly limbic and related structures, including the piriform cortex. This

phenomenon is not observed immediately following induction and takes as much as a month to develop. Repeated trials of rewarding LH stimulation often leads to the development of overt seizures (Nobrega et al., 1993). In our study, while the animals did not exhibit any such signs during the few behavioural tests conducted pre-lesion, it is unknown whether further stimulation exposure would have induced a kindling effect, as is frequently the case. Thus, one possibility is that the decrease in metabolic activity observed in the piriform cortex is related to the removal of a focal epileptogenic site. The second, given the role of the piriform cortex in processing olfactory input, may reflect damage to olfactory elements recruited by LH stimulation.

Recently, there has been much interest in the role of the central extended amygdala as the source of the projection that descends to medial forebrain bundle sites that mediate BSR (Koob, 1999). Although our data implicate the cortical sub-nucleus of the amygdala more than components of the central extended amygdala, there are nonetheless important intrinsic connections (De Olmos and Heimer, 1999) between these two areas that probably integrate signals involved in various aspects of emotional behaviour (Savander et al., 1996). The specific function of individual sub-nuclei of the amygdala, pertinent to this discussion, the posterolateral and posteromedial nuclei, is poorly understood. The results of this study have oriented our future efforts towards untangling the contributions of particular amygdaloid sub-structures in modulating LH self-stimulation. To this end, we have found that lesions of the cortical nuclei that were identified in this study have a dramatic effect on thresholds for lateral hypothalamic BSR (Bielajew, Miguelez and Shiao, 2002), increasing the values by as much as 100%, and the opposite effect on thresholds derived from rewarding ventral tegmental area stimulation (Bielajew, Miguelez and Shiao, 2002). Interestingly, while both sites have been shown to have

direct functional connections with respect to reward (Bielajew and Shizgal, 1986; Murray and Shizgal, 1996a and 1996b; Shizgal et al., 1980), each appears to have a unique relationship with the amygdala.

Given the finding that lesions of the LH produce metabolic alterations in the cortical sub-nuclei of the amygdala, we decided to behaviourally test the functional links shared by these two sites with respect to reward in the second investigation of this thesis. In addition, we also attempted to unveil potential modulatory influences of the amygdaloid complex on BSR elicited from the VTA. Finally, a third variable of interest was added in that we assessed whether the cross-talk between the MFB and the amygdaloid complex with respect to reward occurs interhemispherically.

EXPERIMENT 2

Published as: Bielajew, C., **Miguelez, M.**, Shiao, R. (2002). Electrolytic lesions of the cortical and adjacent nuclei in the amygdala differentially influence thresholds for rewarding medial forebrain bundle stimulation. **Behavioral Neuroscience, 116,** 660-671.

1. Abstract

Following electrolytic lesions to the cortical and adjacent amygdaloid sub-nuclei, thresholds for rewarding medial forebrain bundle (MFB) stimulation were tracked in19 rats with bilateral and eight with single implants. Results were categorized into three groups, depending on the magnitude of the lesion effect on ipsilateral frequency thresholds - either substantial (more than 60%), small (more than 26%), or none (less than 26%), compared to baseline values. Five animals exhibited threshold increases up to 225%, among the most considerable reported to our knowledge. Small shifts were observed in another five animals, and no change in the remaining 17 rats. In addition, threshold changes in the contralateral electrode mirrored ipsilateral ones. These results suggest that specific amygdaloid sub-nuclei modulate MFB reward signals through a diffuse collateralized organization of fibers and lend support to the existence of interhemispheric links in MFB reward pathways.

2. Introduction

In the last two decades, much progress has been made towards the characterization of the substrate mediating brain stimulation reward (BSR). At the heart of this effort is the medial forebrain bundle (MFB), a site from which robust BSR can be effortlessly obtained. Based on behavioural adaptations of classical electrophysiological paradigms, it is known that the small myelinated axons that carry the reward signal course uninterrupted between structures that lie along the MFB. Their rostral to caudal progression includes the lateral preoptic area, lateral hypothalamus (LH), ventral tegmental area (VTA), and periaqueductal gray (Bielajew et al., 2000, 2001; Bielajew and Shizgal, 1982, 1986; Boye and Rompré, 1996; Durivage and

Miliaressis, 1987; Gratton and Wise, 1988; Shizgal et al., 1980).

While this work has concentrated on elaborating the functional map of MFB components, there are many extra-MFB sites that mediate BSR and contribute projections to this pathway. Among these, the amygdala (Paganis et al., 1995; Touzani and Velley, 1998) is of particular interest given its major role in various motivational processes. Amygdaloid structures that participate in producing and maintaining addiction are believed to be part of the same circuit that supports BSR in the basal forebrain (Koob, 1999). Additionally, the "extended" amygdala, coined by Johnston (1923), which comprises the central amygdaloid nucleus, the dorsal substantia innominata, and the bed nucleus of the stria terminalis (Cassell, Freedman and Shi, 1999), is thought to be a sensory-reward association area. Finally, the amygdaloid complex constitutes a source of connections to the hypothalamus and the VTA, via two compact bundles of fibers: the stria terminalis (Han and Ju, 1990; Prewitt and Herman, 1998) and the ventral amygdalofugal pathway (Prewitt and Herman, 1998).

Lesion techniques have been exploited to study the relationship between the MFB and more distal structures. However, this strategy, with few exceptions, has not been applied to evaluating the contribution of the amygdala to rewarding MFB stimulation. What is now considered a classic study is the work by Olds and Olds (1969) who found that lesions anterior to MFB self-stimulation sites, a few of which encompassed large areas of the amygdala, tended to be more effective in reducing response rates than damage placed more posteriorly. More recently, Warazcynski, Ng Cheong Ton and Shizgal (1990) found little or no alteration in the thresholds for LH and VTA self-stimulation following electrolytic lesions of the principal amygdaloid sources of descending MFB fibers - the amygdalofugal pathway, and the medial and

basolateral sub-nuclei. In a recent investigation using cytochrome oxidase histochemistry - a metabolic method developed to investigate long-term alterations in functional activity (Wong-Riley, 1989; Hevner, Liu, and Wong-Riley, 1995) - we found support for the involvement of specific amygdaloid sub-nuclei in BSR. Electrolytic lesions of rewarding LH loci significantly reduced oxidative metabolism, principally in the cortical amygdaloid nuclei, and to a lesser extent, in the piriform cortex (Miguelez et al., 2001). Thus, based on anatomical, metabolic, and behavioural data, we decided to explore this question by producing lesions in or around the cortical amygdaloid nuclei and to evaluate the effects on a continuum of MFB structures, from the LH to the VTA.

3. Methods

3.1 Subjects and surgery

Stereotaxic surgery was performed on 30 male Long-Evans rats (Charles River Canada, St-Constant, Québec, Canada), under deep anaesthesia induced by an intraperitoneal injection of 65 mg/kg of sodium pentobarbital (Somnotol); the rats' weights at surgery ranged from 300 to 400 g. Stainless steel monopolar electrodes were bilaterally aimed at the cortical nuclei of the amygdala and along the MFB to target the LH and the VTA. Thus, each rat received two MFB and two amygdala implants. The stereotaxic coordinates, based on the Paxinos and Watson (1986) atlas, were as follows: Amygdala - 2.55 mm posterior to bregma, 3.8 to 4.1 mm lateral to the midsagittal suture, and 9.6 to 9.8 mm below the skull surface measured at bregma; MFB - from 3.0 mm to 4.8 mm posterior to bregma, 0.7 to 1.6 mm lateral to the midsagittal suture, and 8.3 to 8.4 mm below the skull surface measured at bregma. The rats were allowed one week

recovery from surgery. They were housed in separate cages and kept on a 12-h light/dark cycle with light onset at 1900 h. Purina rat chow and water were available ad libitum; food consumption and body weight were recorded two to three times per week.

3.2 Screening for self-stimulation

Conventional shaping procedures were used to screen the rats for self-stimulation at each of the pair of bilateral MFB electrodes. Tests were conducted in Plexiglas operant chambers (28.6 x 32.6 x 30.6 cm) equipped with a lever on the right wall, located 3 cm above the floor. Each lever press produced a 0.5 s train of monophasic cathodal rectangular pulses of 0.1 ms duration with the frequency fixed at 40 Hz; the currents ranged from 100 μA to 800 μA. Stimulation was supplied by constant-current amplifiers (Mundl, 1980) and integrated circuit pulse generators, built in-house. In order to avoid the build-up of charge at the electrode/tissue interface, the current between pulses was shunted to ground via a low resistance pathway. The current was continuously monitored on an oscilloscope by reading the voltage drop across a 1 kΩ precision resistor in series with the rat.

Only animals showing robust responding for MFB stimulation (more than 30 bar presses per minute) from at least one of the two electrodes were retained for the experiment. Once reliable responding was observed, frequency thresholds were determined by collecting rate-frequency curves every day at two current values; the first current selected was the lowest one to support consistent self-stimulation rates and the second was 0.2 $\log_{10}$ units greater than the first (for example, 200 and 320 μA). Rate-frequency curves were obtained by conducting a series of 60 s trials, during which the current was held constant and the frequency of pulses was reduced in

0.1 $\log_{10}$ steps each trial from a value that produced the highest rate of bar-pressing to one that generated few or no responses. Typically a sequence of four to five frequencies were necessary to observe this pattern. Thresholds were interpolated from the rate-frequency function by locating the frequency value associated with 50% of the maximum rate. The curves were generated daily until the threshold values did not vary by more than 0.05 $\log_{10}$ units for three consecutive days.

All behaviours resulting from amygdaloid stimulation, including bar-pressing, were recorded; no threshold determinations were conducted at this site. The purpose was simply to document whether the site supported self-stimulation and any other obvious stimulation bound behaviours.

Once MFB threshold values were deemed stable according to the above criterion, rats were anaesthetised with the inhalant Halothane and a unilateral electrolytic lesion was applied to the amygdaloid site ipsilateral to the MFB electrode associated with the lowest frequency threshold; damage was induced via a 20 s anodal current set at 6.0 volts. Recall that all animals had bilateral implants, of which one or both electrodes were used. Following this procedure, MFB frequency thresholds were assessed the next day and thereafter every second day for a total of three to four weeks.

3.3 Histology

After completing all behavioural tests, subjects were perfused intracardially with an initial saline wash followed by a solution of 10% formalin. Brains were extracted and stored in 10% formalin until sectioned (30 μm) and stained with Nissl in order to verify the location of the

electrode tips.

4. Results

Stable threshold values were obtained from both electrodes in 19 of 30 rats. Another 8 animals met the criterion for stability in one of the pair of MFB electrodes. Three rats were removed from the study due to unreliable rates of self-stimulation from both electrodes. Although frequency thresholds were always determined at two current levels, the lowest one to support stable thresholds and a second value, 0.2 $\log_{10}$ units higher, there was no significant difference in the pattern of post-lesion threshold changes at the two currents. Consequently, we report only the data associated with the lower of the two values. The average low current and associated standard error of the mean for each group (see group designation below) and side were 124±15 μA (Group A - ipsilateral), 140±20 μA (Group A - contralateral), 178±18 μA (Group B - ipsilateral and contralateral), 190±13 μA (Group C - ipsilateral) and 257±62 μA (Group C - contralateral).

Following the amygdaloid lesion, MFB threshold shifts that were obtained on the side ipsilateral to the lesion were categorized using an adaptation of the scheme adopted by Arvanitogiannis, Waraczynski, and Shizgal (1996). Accordingly, Group A included rats exhibiting substantial shifts in thresholds ($\rangle$ 60% or 0.20 $\log_{10}$ units), Group B, animals showing small threshold shifts (between 26 and 60% or from 0.10 to 0.20 $\log_{10}$ units), and Group C, rats showing no change ($\langle$26% or 0.10 $\log_{10}$ units) in thresholds. Thus, five rats exhibited substantial threshold shifts (Group A), another five showed smaller post-lesion threshold shifts (Group B), and the remaining 17 rats were categorized as having no threshold shifts (Group C) on the MFB

electrode ipsilateral to the amygdaloid lesion.

The group designation of the animals was determined by two independent judges. In order to evaluate their measure of agreement while correcting for chance, Cohen's Kappa coefficient (Howell, 1992) was computed, yielding a coefficient of $\kappa = 0.67$ (representing an inter-judge agreement on 93% of the cases). As expected, the few disagreements occurred for cases in which threshold changes straddled two categories very closely - the small and no change groups. The disagreements were resolved by determining the median value across sessions and placing the animal in the corresponding group.

Figure 11 shows the location of the amygdaloid lesion in all animals, Figure 12, the location of the bilateral implants for individual Group A and Group B rats, and Figure 13, electrode placements of Group C rats. Although we observed no particular differences between groups with respect to electrode placement, rats in Group C generally had a higher concentration of posterior lesions, while Group A's lesions were predominantly anterior. In the few cases where Groups A and C lesions overlapped in AP location (for example, rats 83 from Group A and rats 80, 95 and 96 from Group B, A's at -2.30 posterior to bregma), A's tended to be a bit more medial than C's. In all animals except one (R83), the lesion encompassed at least one of the major cortical nuclei of the amygdala (anterior, posteromedial and/or posterolateral) as well as, in some cases, adjacent amygdaloid structures such as the basolateral amygdaloid nucleus. The 27 ipsilateral electrode placements were scattered along the MFB, rostrally from the reticular thalamic nucleus and caudally to the parabrachial pigmented nucleus, with the majority of placements located in or around the LH and the VTA. Within each animal, there appeared to be almost perfect bilateral electrode alignment.

Figure 14 shows the percentage change in thresholds from baseline following the amygdaloid lesion for rats in Group A; data obtained from the ipsilateral electrode are shown on the left and the corresponding contralateral findings on the right. In all cases, the amygdaloid lesion gave rise to increased frequency thresholds (interpreted as a reduction in the stimulation rewarding value) throughout the post-lesion testing period, exceeding as much as a 200% shift in baseline values by the 28th day following the lesion. In the three animals in this group with a contralateral electrode, a similar pattern was observed, although these increases were comparatively smaller, generally around 50%.

In Figure 15, the results obtained from animals in Group B (shifts ranging from 26 to 60%) are shown. In contrast to the consistent pattern observed in Group A (substantial shifts), frequency thresholds in the rats consigned to this group either increased (R90 and R92) or decreased (R72, R88, and R89). However, the individual patterns were reliable over session, achieving shifts of roughly 50% from baseline across animals. Similar to our observation in Group A, contralateral changes tended to be smaller than the corresponding ipsilateral ones.

The average threshold shift post-lesion obtained in each group is shown in Figure 16. Note that the absence of threshold shifts suggested by the data associated with Group B reflects the divergent patterns exhibited by these animals - evident in the individual graphs (Figure 15). Again, animals with electrodes in which no threshold changes were observed (Group C) exhibited the same pattern regardless of which electrode was tested - ipsilateral or contralateral.

Figure 11. Three sets of anatomical figures showing the location of the lesion in all subjects (N = 27). The plates in this and the following anatomical figures are based on tracings from the Paxinos and Watson atlas (1986). Group A includes rats that showed a substantial (more than 0.20 $\log_{10}$ units change from baseline) lesion-induced shift in frequency threshold, Group B - a small (0.10 to 0.20 $\log_{10}$ units) shift, and Group C - no shift (less than 0.10 $\log_{10}$ units). In all groups, the size of the shift is based on the data obtained from the electrode ipsilateral to the amygdaloid lesion. The tracings for each group are ordered from left to right, beginning with the most anterior site. The alphanumeric that appears at the top of each drawing identifies the subject and the number located at the bottom refers to the millimetric distance behind bregma associated with that drawing.

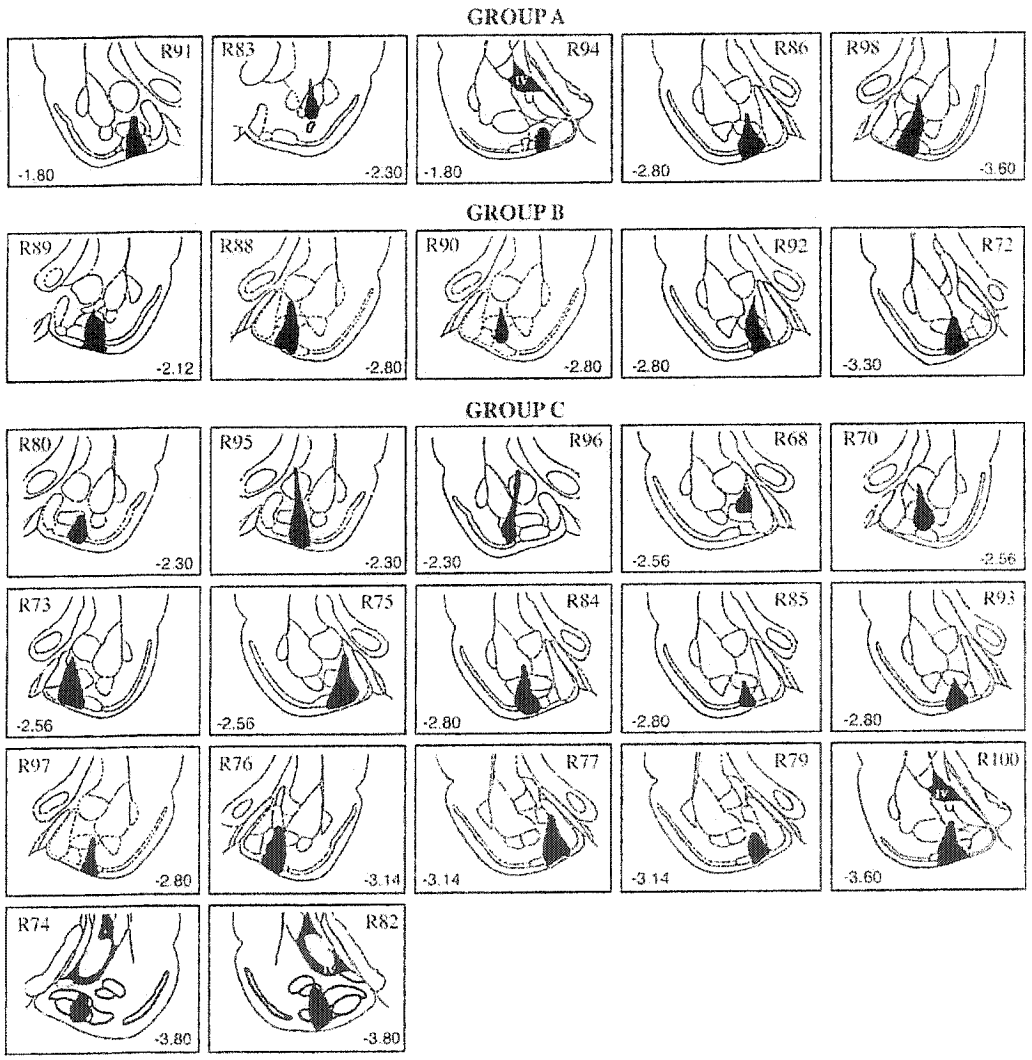


Figure 12. Location of bilateral electrode tips (filled circles) for rats showing a substantial (Group A) and small (Group B) lesion-induced shift in frequency threshold. Recall that subjects were included in the first group (A) if the shift was rated as greater than a 0.20 $\log_{10}$ change from the baseline frequency threshold and in the second group (B) for changes greater than 0.10 $\log_{10}$. In both cases, it is the data derived from the electrode ipsilateral to the lesion that determines group designation. For each group, the tracings are arranged from top to bottom, beginning with the most anterior; ipsilateral electrode tips appear on the left and the corresponding contralateral tip for that animal, if available, on the right. The rat identification is shown at the top of the plate(s) and the plate's location (relative to bregma) is indicated on the bottom.

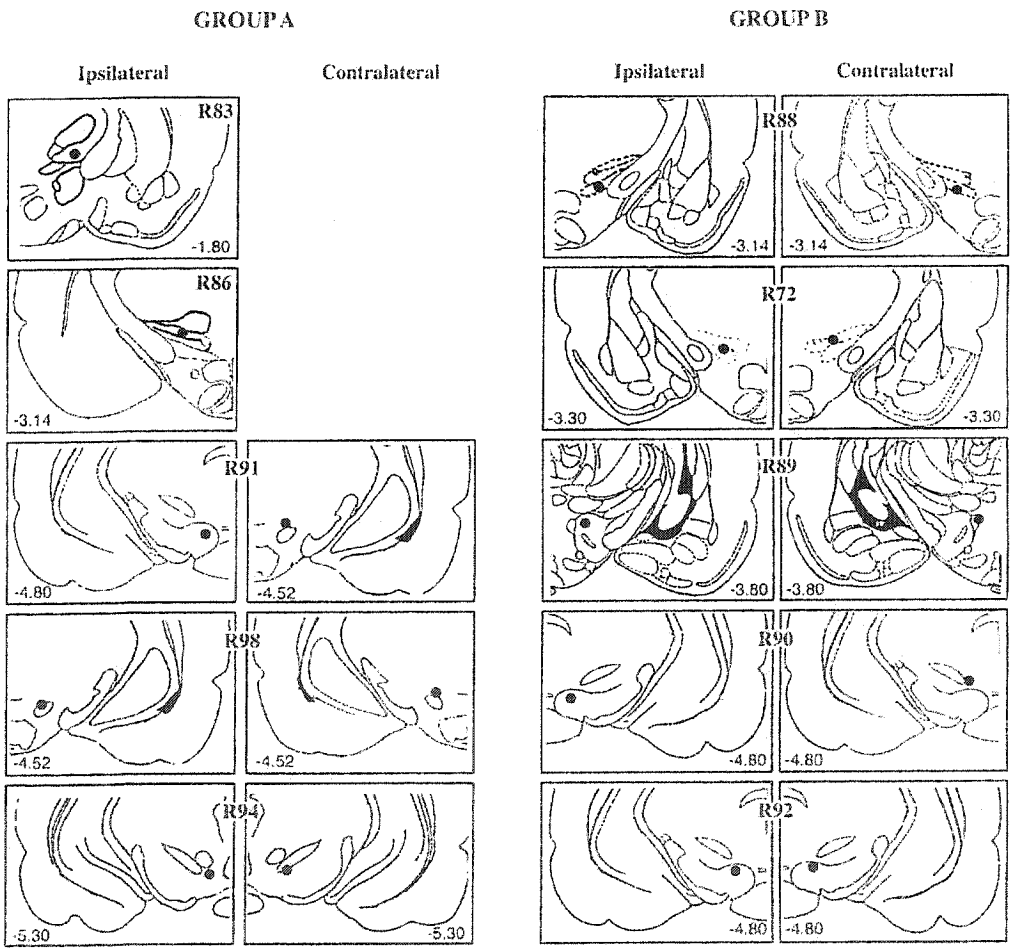


Figure 13. Location of the bilateral electrode tips (filled circles) associate with the animals that exhibited no lesion-induced shift in frequency threshold (less than a 0.10 $\log_{10}$ change from the baseline value) - Group C. The placements are arranged from top to bottom in anterioposterior order. The number on the left of each tracing refers to that plate's millimetric distance behind bregma.

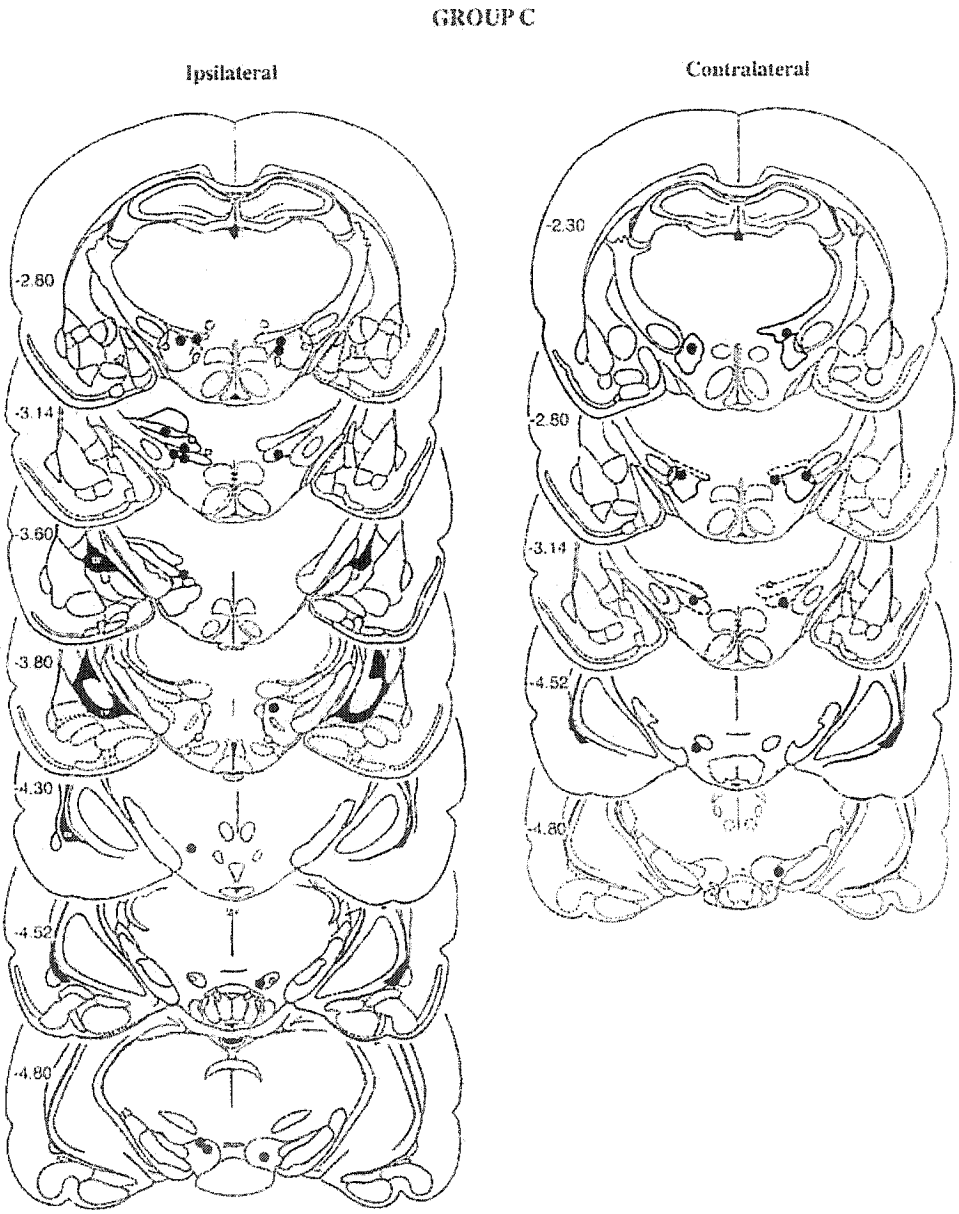


Figure 14. The shift in frequency threshold, expressed as % change from threshold over time, is shown for the individual animals in Group A, with the thresholds obtained from the ipsilateral electrode plotted on the left and the corresponding contralateral electrode results on the right. Recall that the data obtained from the electrode ipsilateral to the lesion determines group designation - in this case, a change greater than 0.2 $\log_{10}$ units. Note that no contralateral data are available for subjects R83 and R86. The baseline is represented by the horizontal line anchored at 0. The alphanumeric identified the rat and below it appears the anterioposterior location of the electrode tips, according to Paxinos and Watson (1986). Upward shifts (above 0) indicate an increase in the frequency threshold which is interpreted as a reduction in the rewarding effect and downward shifts, the reverse - a decrease in the frequency threshold and therefore greater reward.

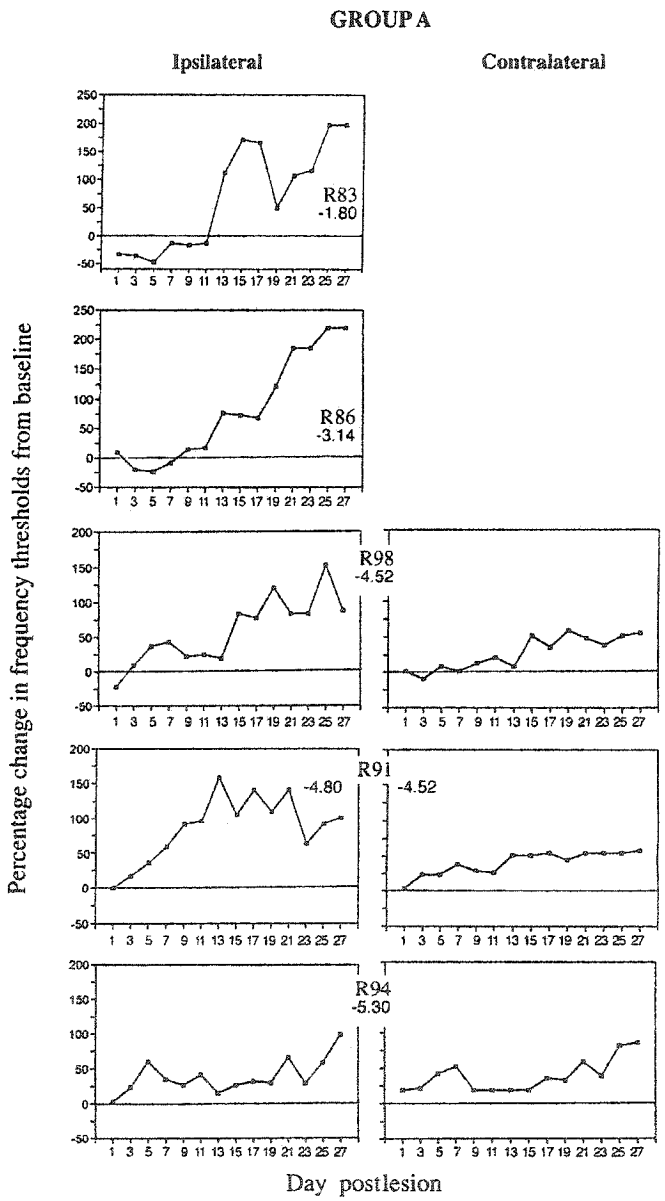


Figure 15. The shift in frequency threshold, expressed as % change from threshold over time, is shown for the individual animals in Group B, with the thresholds obtained from the ipsilateral electrode plotted on the left and the corresponding contralateral electrode results on the right. Recall that the data obtained from the electrode ipsilateral to the lesion determines group designation - in this case, a change rated at between 0.10 $\log_{10}$ and 0.20 $\log_{10}$ units greater than the baseline threshold value. The baseline is represented by the horizontal line anchored at 0. The alphanumeric identified the rat and below it appears the anteroposterior location of the electrode tip according to Paxinos and Watson (1986). Upward shifts (above 0) indicate and increase in the frequency threshold which is interpreted as a reduction in the rewarding effect and downward shifts the reverse - a decrease in the frequency threshold and therefore greater reward.

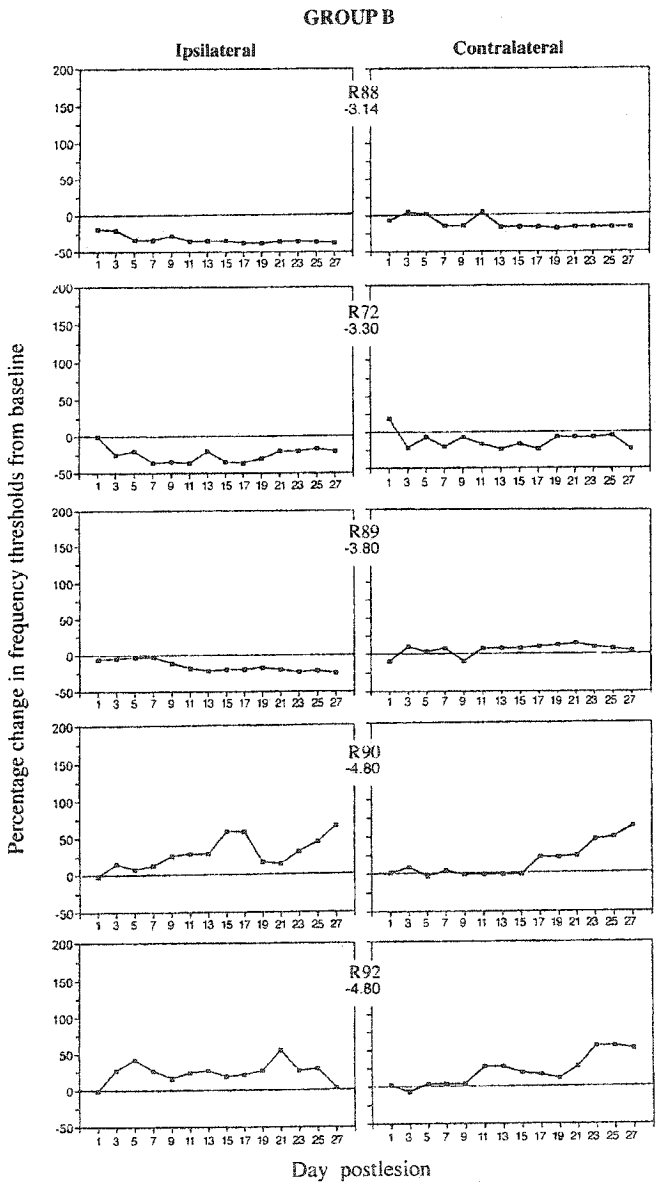
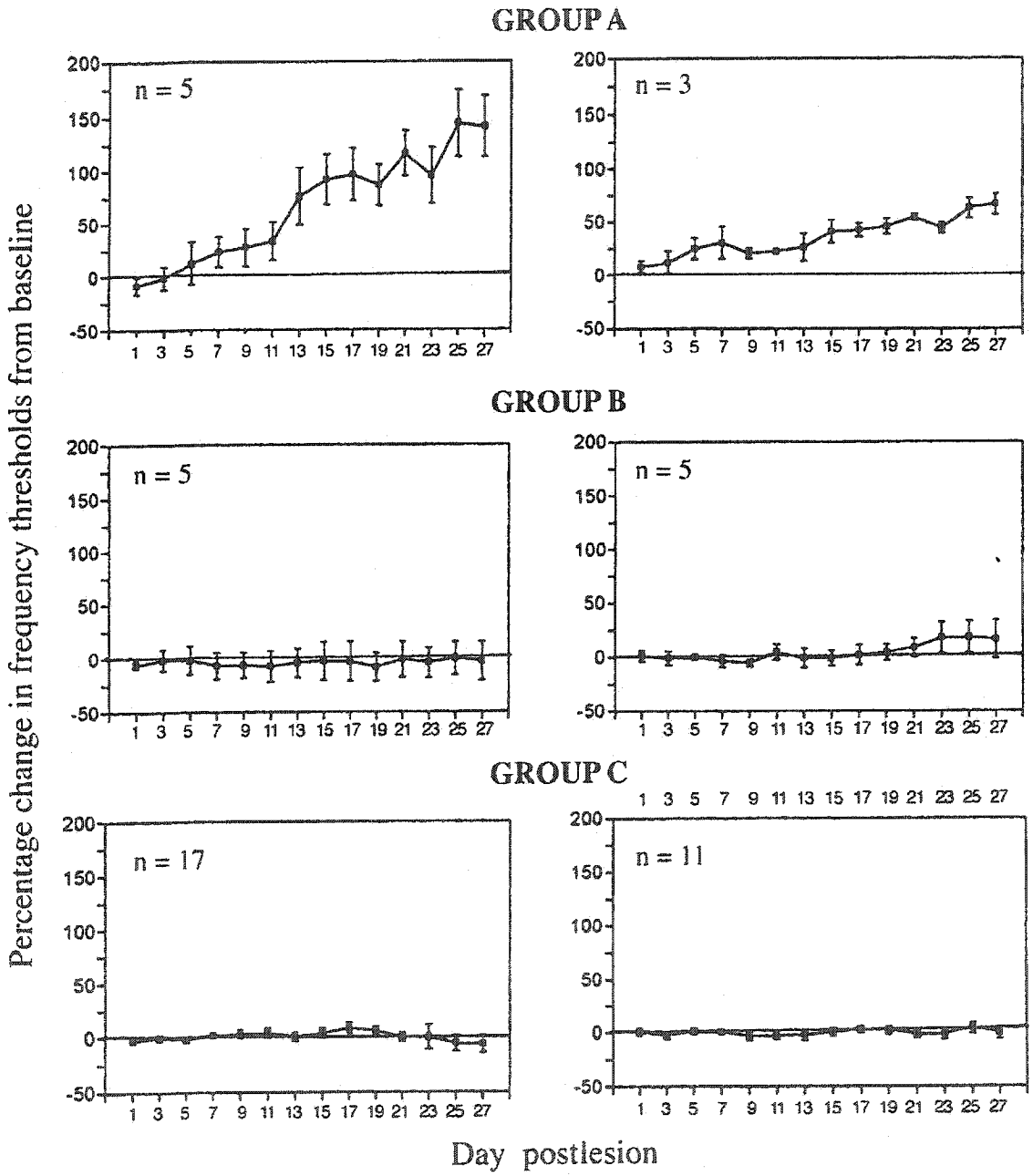


Figure 16. The averaged lesion-induced threshold changes for each of the three groups. Note that the ipsilateral data for Group B appear relatively flat because two of the subjects exhibited shifts above and three below baseline.



5. Discussion

In the present study, the effects of amygdaloid lesions were categorized into three groups, based on post-lesion threshold differences (substantial, small, or none) from baseline threshold. The substantial changes exhibited by five animals are among the largest post-lesion decreases in rewarding efficacy ever reported to our knowledge - ranging from 60% to as much as 225%. Animals displaying smaller post-lesion threshold changes (from 26% to 60%) were not consistent in direction, with two showing an increase and three, a decrease in threshold value relative to baseline performance[1]. The remaining seventeen animals showed no threshold shifts.

We did examine whether electrode placement could account for the differences in magnitude of threshold shift across animals, and found nothing illuminating in that respect that would predict group designation. Also, in all but one case (R82), lesions were located either rostral to or at the same level as the electrode placement, and the stimulation parameter values were similar across groups as were baseline rates and thresholds. However, subtle differences in lesion placement were detected: damage produced in rats exhibiting substantial shifts in thresholds (Group A) was generally more anterior to that of animals showing no change in thresholds (Group C). This observation corresponds well with what is known about the anatomical organization of axonal connections linking the amygdaloid complex with the MFB in that a predominant proportion of the amygdaloid sub-nuclei projections to the hypothalamus travel through the bed nucleus of the stria terminalis (McDonald, 1998; Price, Russchen, and

---

[1] Note that this difference visually translates into a relatively flat line when computing average threshold values across animals (see Figure 16, Group B, left-hand panel). When considering data from this group, it is thus more meaningful to study the individual profiles, depicted in Figure15.

Amaral, 1982).

In the few cases where Group A and C lesions had anterior-posterior overlap, Group A damage tended to be more medial than Group C's, suggesting that the former may have been more likely to inactivate a higher concentration of axons exiting the amygdala to join the MFB. In support of this hypothesis, anatomical data indicates that the amygdaloid nuclei which are located at the most medial portion of the complex project not only to the medial part of the bed nucleus of the stria terminalis (which is, as mentioned above, an important relay station for fibers travelling to the MFB), but also more directly to the medial amygdaloid nuclei and the hypothalamus (Eiden et al., 1985; Kawata et al., 1985). Although the nuclei that contribute most fibers to the lateral hypothalamus (either directly or through the stria terminalis) are the basal (basolateral) and central amygdaloid nuclei, these structures share intrinsic connections with the cortical nuclei of the amygdala (McDonald, 1998; Price, Russchen, and Amaral, 1982). Consequently, it is possible that the target areas of the present study, the cortical and adjacent nuclei of the amygdala, exerted their modulatory effect on MFB reward indirectly through the various "relay stations" that comprise the extensive intra-amygdaloid connections.

The view that the amygdaloid sub-regions need to be considered separately when studying them in the context of MFB reward pathways is supported by the findings of Jackson and Gardner (1974) who reported that stimulation of the basolateral amygdala had no impact on LH self-stimulation rates, whereas corticomedial amygdaloid nucleus stimulation inhibited them. This early work is one of few to demonstrate the specificity of amygdaloid subregions in modulating reward derived from MFB activation.

In a study similar to this one, Waraczynski, Ng Cheong Tom and Shizgal (1990) observed

that threshold values for rewarding LH as well as VTA stimulation in nine rats remained close to baseline levels following electrolytic lesions of the amygdaloid complex, specifically the amygdalofugal pathway, and the medial and basolateral sub-nuclei, which are located in or around the central division of the amygdaloid complex. Our lesions, on the other hand, caused damage primarily but not exclusively to cortical sub-nuclei. Thus, it may be these particular sub-nuclei that are critical to ensuring the integrity of the MFB reward signal generated at these sites. The problem with this idea is the fact that one animal (R83) that exhibited a substantial post-lesion change in threshold had damage that did not include the cortical amygdaloid nuclei. Perhaps degenerative changes affecting non-cortical amygdaloid neurons following the lesion extended to cortical cells via the process of transneural degeneration (Aguayo et al., 1990 a and b, Kandel, Schwartz, and Jessell, 1991). Note that this particular animal differed from the others in having the most anterior lesion and electrode placements, so that the explanation for his threshold change may involve a unique anatomical arrangement.

Another element to consider is the fact that our lesions sometimes encompassed not only the cortical nuclei, but also, in most cases, the basolateral amygdaloid nucleus to some degree. As mentioned previously, this structure contributes a major proportion of the fibers to the MFB (Price, Russchen, and Amaral, 1982), so that a lesion comprising even a small portion of this nucleus could presumably affect signals being carried to the hypothalamus, and by extension, to the VTA, independently from the damage caused to the cortical nuclei. This explanation, however, is not consistent with previous findings of our laboratory, in which we evaluated post-lesion metabolic changes using cytochrome oxidase histochemistry (Miguelez et al., 2001). Following damage to rewarding LH sites, the density of reaction product was significantly

decreased in the cortical region of the amygdala and somewhat in the piriform cortex, but not in other sub-nuclei, including the basolateral one. These changes were apparent two to four weeks post-lesion, as expected given that this marker measures long-term patterns of neuronal activity (Wong-Riley, 1989).

Nonetheless, in other stimulation studies in which metabolic changes were used to evaluate the relationship between the MFB and the amygdaloid sub-nuclei, results suggest that the basolateral and central amygdaloid nuclei are activated following rewarding MFB stimulation, as measured by other markers such as 2-DG (Esposito et al., 1984; Porrino et al., 1984; Yadin, Guarini, and Gallistel, 1983) and c-fos (Hunt and McGregor, 1998). Note that such markers are more sensitive to immediate metabolic demands, as opposed to cytochrome oxidase which tracks perdurable neuronal activity (Wong-Riley, 1989). This apparent discrepancy may be reconciled by the suggestion that lesions specifically aimed at the cortical amygdaloid nuclei may induce a progressive orthograde degeneration along relevant axons descending from the amygdala past the MFB stimulation sites. Although to our knowledge, the specific characteristics of axonal degeneration in the amygdala have not yet been described, there is some indication that within the central nervous system, the onset of synaptic transmission failure is highly variable depending on the exact location of axonal injury. Synaptic transmission of the distal segment will initially be less disrupted following lesions placed closer to the cell body than the synaptic terminal. In fact, if the distal segment is sufficiently long, axonal transport and synaptic transmission can continue for some time. Transmission changes in the axonal distal segment emerge about one week after the terminals begin to degenerate and continue over the next few months until complete degeneration of the distal segment (Aguayo et al., 1990 a and b, Kandel,

Schwartz, and Jessell, 1991).

Perhaps this phenomenon accounts for the delay (approximately one week) in threshold changes observed in some of our animals categorized with substantial and small changes, and the lesion data in other similar investigations (Gallistel, Leon, and Waraczynski, 1996; Waraczynski and Shizgal, 1995). In this study, the three of five substantial threshold shifts that were observed were evident one day post-lesion and thereafter increased progressively. Changes in the remaining two cases, in contrast, were first detected no earlier than one week post-lesion.

From our understanding, this variability in the time course of lesion effects occurs irrespective of the self-stimulation site, lesion site, or lesion technique employed. For instance, in Waraczynski and Shizgal's (1995) investigation of the effects of electrolytic lesions to the parabrachial region on LH and VTA reward thresholds, two rats showed an immediate and sustained effect, three rats had progressive increases in frequency thresholds over the two week test period, and two showed effects only five days post-lesion. Similarly in Gallistel, Waraczynski and Shizgal's (1996) study of the impact of electrolytic lesions and knife-cuts contralateral (caudal) to MFB homologous self-stimulation sites, the electrolytic lesions generated an immediate increase in threshold in two animals, a progressive one in seven, and no change in one until the ninth day post-lesion. The knife-cuts produced an immediate threshold change in six animals and no change in one until the tenth day post-lesion. Finally, Acheson, Waraczynski, and Perkins (2000) found that following bilateral electrolytic lesions of the motor nucleus of the trigeminal nerve, MFB thresholds were markedly increased in three animals; however, these effects were evident only one week after the injury. These few examples illustrate the variety of patterns observed in MFB thresholds following central nervous system damage and

the difficulty in predicting the time-course of lesion effects.

The application of lesions as a strategy for mapping the circuitry underlying BSR has had a controversial history. The main problem has been that similarly placed lesions do not produce consistent effects on thresholds for rewarding MFB stimulation (see Simmons, Ackerman, and Gallistel, 1998). The few studies that report substantial and enduring LH and VTA threshold increases include ones in which damage was induced by N-methyl-D-aspartic acid to the lateral preoptic area (Arvanitogiannis, Waraczynski, and Shizgal, 1996), temporary inactivation via lidocaine to the retrorubral fields (Waraczynski and Perkins, 2000), and electrolytic lesions in caudal MFB (Gallistel et al., 1996). Notwithstanding the peculiarities associated with different lesion techniques, these findings point to structures that are likely critical in carrying and/or modulating MFB reward signals.

In view of our earlier work (Miguelez et al., 2001), we adopted the electrolytic lesion technique to explore the contribution of the cortical and adjacent amygdaloid sub-nuclei to MFB reward circuitry. In this investigation, although subtle differences in lesion placement were detected, which could potentially account for the variability in our data, the exact location of damage could not predict with any accuracy the impact on reward thresholds. How do we interpret this variability observed in the present data and that of others. Recently, Simmons, Ackerman, and Gallistel (1998) have attempted to explain this conundrum using a combination of techniques, including electrolytic lesions, 2-DG, and True Blue retrograde tracer. Their failure to find consistent relationships between the degree of labelling and amount of damage led them to propose that the MFB carries the reward signals via highly collateralized nets of axons.

This explanation could also account for the fact that in our study cortical amygdaloid

damage produced effects ranging from no change in threshold to massive and sustained decreases in the rewarding value of MFB stimulation. While the animals in this study had seemingly similar stimulation and lesions sites, standard histological methods are inadequate for detecting subtle differences in nuclei boundaries; this is particularly true in the case of the amygdaloid complex and its elaborate intrinsic anatomy (McDonald, 1998). Thus, it is conceivable that signals carried between the amygdala and the MFB are conducted via an extensive network of neural elements. This functional arrangement would be highly profitable, considering that the amygdala plays a crucial role in the modulation of various motivational processes crucial for survival-oriented behaviours. For instance, it has been shown that the amygdala alters responses involved in the perception of emotional stimuli (Fellous, 1999; Halgren,1992), the production of emotional responses (Davidson and Irwin, 1999), and the neuronal mechanisms giving rise to responses of fear and anxiety (Charney, Grillon, and Bremner, 1998).

The literature concerning the relationship between the amygdala and the MFB with respect to reward is quite scarce. In their work on LH-amygdaloid interactions, Kane and his colleagues (1991) examined whether pairing trains of rewarding LH and amygdala stimulation altered the thresholds obtained from stimulation at either site alone. They found no significant summation of paired-pulse stimulation regardless of the order of pulse presentation when the currents associated with the two sites were selected to produce similar frequency thresholds. A much earlier study (Szabo et al., 1972) in which trains of pulse pairs comprising LH pulses of variable current and fixed amygdala ones (just above threshold) yielded relatively minor improvements in rate and threshold compared to trains of LH pulses alone. The LH paired with other sites, the medial prefrontal cortex for example, yields similar results (Schenk and Shizgal,

1982). This phenomenon, poor spatial summation, even occurs occasionally with bilateral stimulation of homologous MFB structures (Malette and Miliaressis, 1995; Shizgal et al., 1980). Thus, lack of significant summation may not be indicative of the nature of the relationship between two functionally related sites.

In the present study, there was good agreement between ipsi- and contralateral BSR thresholds following the lesion. Effects on contralateral thresholds were either of the same magnitude or a little less than that observed ipsilaterally. This could not reflect large differences in the rewarding strength of the electrode pair given that the lowest current on average to support self-stimulation was similar on both sides regardless of group; the only tendency was for substantial and small changes (Groups A and B respectively) to be associated with sites in which the lowest currents to support BSR were required. Given the number of animals used in this study, there was surprisingly good bilateral electrode alignment as confirmed by our histology, a factor that may explain why our ipsilateral and contralateral lesion effects were so similar. This phenomenon - bilateral effects of unilateral lesions - has not been well-examined. In the few cases where interhemispheric threshold differences have been documented by others, post-lesion alterations in ipsilateral thresholds were generally not accompanied by similar changes on the contralateral side (Waraczynski and Perkins, 1998, 2000; Whitehurst, 2000); lesion changes were usually restricted to structures located in the damaged hemisphere.

There are at least two different possibilities to explain our contralateral data. The first is that reward fibers communicate their signals bilaterally at the level of the MFB. This view is endorsed by the results of a recent study (Malette and Miliaressis, 1995), in which interhemispheric interactions between bilateral self-stimulation sites in the MFB were

investigated using a paired-pulse stimulation technique. Evidence was found for direct axonal and indirect synaptic connectivity in roughly 60% of the 101 pairs of target stimulation sites, suggesting that the substrate for MFB reward may include, to a large extent, decussating neurons that insure links between both hemispheres. However their model has been questioned by Shizgal (1997), who suggests an alternative explanation for their data.

A second possibility is that the modulation of MFB thresholds contralateral to the amygdaloid lesion may not be related to the integrity of the ipsilateral amygdala-MFB connection but instead reflect input from the contralateral (undamaged) link. If the weight of this signal were sufficiently large, it could compensate for any loss of rewarding impact due to the injury incurred on the ipsilateral side. Both these explanations have anatomical validity, since most of the interhemispheric connections between amygdaloid complexes are achieved through fibers that relay in the stria terminalis and then cross over in the anterior commissure (Price, Russchen, and Amaral, 1982). Since our lesions were located before the stria terminalis, either communication route would explain the similarities between our ipsi and contralateral post-lesion changes in thresholds, that is, ipsilateral amygdala ➡ ipsilateral MFB ➡ contralateral MFB or ipsilateral amygdala ➡ contralateral amygdala ➡ contralateral MFB. We would need to explore the effect of serial cortical damage on bilateral MFB thresholds in order to resolve this question.

Finally, we recognize that whatever role the cortical nuclei play in modulating BSR, it is likely accomplished via intra-amygdaloid connections with the extended amygdala, an idea which is anatomically viable (De Olmos and Heimer, 1999). The extended amygdala is gaining importance in recent years as a key structure in the development of drug reinforcement and addiction, which along with BSR are thought to be mediated by the same underlying MFB

circuitry (Koob, 1998, 1999). However, our data suggests that attention should be directed towards an examination of the specific contribution of the cortical sub-nuclei with respect to reward derived from activation of the MFB.

The data presented in Experiment 3 confirmed that the amygdaloid complex alters reward signals elicited from a continuum of MFB sites coursing from the LH to the VTA. Furthermore, it was shown that such a modulation takes place in both hemispheres of the brain. The final thesis experiment aimed at refining the target sub-nuclei of the amygdala involved in this cross-talk as well as the nature of interhemispheric communication in this reward-relevant pathway.

EXPERIMENT 3

Miguelez, M., Kentner, A., Deslauriers, K., Parkinson, M., Fouriezos, G., Bielajew, C. Interhemispheric involvement of the anterior cortical nuclei of the amygdala in rewarding brain stimulation. **In Review for Brain Research.**

1. Abstract

The amygdaloid complex is one of the structures thought to modulate brain stimulation reward (BSR) elicited from the median forebrain bundle (MFB). Previous metabolic and behavioral data from our laboratory point to the amygdaloid cortical nuclei as key to this process. In this study, thresholds for rewarding stimulation of the MFB were determined for forty-two days, twenty-one following an electrolytic lesion to amygdaloid nuclei ipsilateral to the stimulation electrode, and twenty-one following one applied to the contralateral amygdala. A subset of animals showed post-lesion changes in MFB frequency thresholds that were maintained if not augmented after the second lesion. These ranged from 26 to 150% compared to baseline values, among the largest ever reported to our knowledge. Interestingly, damage to anterior sites within the cortical nuclei was the most effective in producing modifications to the rewarding value of the stimulation. Equally singular was the finding that contralateral lesions tended to alter thresholds more than ipsilateral ones, confirming our earlier finding of interhemispheric connectivity in amygdaloid modulation of MFB reward signals. This interpretation was substantiated by tracking long-term metabolic activity in the amygdala using cytochrome oxidase histochemistry. The density of reaction product at damaged amygdala sites was negatively correlated ($r = -0.90$) with the increases in thresholds obtained at contralateral MFB loci. Together with the fact that such large lesion effects are seldom obtained, our metabolic results point to the existence of a relationship between these nuclei and reward signals generated at the MFB. Moreover, our data suggest that this communication takes place interhemispherically.

2. Introduction

The median forebrain bundle (MFB) is made up of as many as 50 fiber collections of different lengths, origins, and destinations (Nieuwenhuys, Geeraedts, and Veening, 1982; Veening et al., 1982). Because of the ease with which brain stimulation reward (BSR) can be elicited from it, this bundle has become a major focus for the study of the neural circuitry underlying goal-oriented behaviours (see Stellar and Stellar, 1985). Although structures that are part of the MFB have been at the heart of the majority of BSR investigations, some loci located outside of this bundle are known to modulate BSR elicited from the MFB (see for example Bielajew and Trzcinska, 1998; Simmons, Ackermann, and Gallistel, 1998).

Among these sites, the amygdala (also called amygdaloid complex) is of primary interest due to its anatomical and functional characteristics. First, the idea that the amygdaloid complex may modulate MFB reward has anatomical validity; the stria terminalis and the ventral amygdalofugal pathway - two major fiber systems - link the two areas (Han and Ju, 1990; Prewitt and Herman, 1998). Second, many converging lines of evidence clearly implicate the amygdaloid complex in motivation and emotion (see Cardinal et al., 2002; Price et al., 1987). A wide variety of behaviours, ranging from feeding (King et al., 1994, 1996; Nishijo et al., 2000) and sex (Kling and Brothers, 1992) to the mediation of sensory associative learning (Gallagher and Holland, 1992; Wilensky, Schafe, and LeDoux, 2000) has been linked with amygdaloid activity. Recent evidence also points at parts of the amygdaloid complex as determinant structures in the processes underlying drug reinforcement and addiction (Koob, 1998, 1999; Koob and LeMoal, 2001), and some amygdaloid nuclei have been shown to support BSR (Kane, Coulombe, and Miliaressis, 1991a; Panagis et al., 1995; Touzani and Velley, 1998). In addition,

stimulation of rewarding MFB sites has been associated with an increase in cellular activity as measured by the c-Fos marker, in a variety of amygdaloid nuclei (Arvanitogiannis et al., 1996; Hunt and McGregor, 1998; Nakahara et al., 1999). It is thus not unreasonable to speculate that the amygdaloid complex may be involved in mediating the rewarding effects of MFB stimulation.

Metabolic and behavioural evidence support the existence of a functional connection between the amygdaloid complex and the MFB in the context of reward. In the first of a series of studies aimed at exploring this question (Miguelez et al., 2001), we showed that electrolytic lesions of rewarding sites in the lateral hypothalamus (LH) significantly reduced oxidative metabolism in the amygdaloid cortical nuclei, as measured by cytochrome oxidase histochemistry. In a subsequent investigation designed to behaviourally test this link (Bielajew, Miguelez, and Shiao, 2002), we examined the impact of electrolytic lesions to the cortical and adjacent amygdaloid nuclei on BSR thresholds elicited from the LH and ventral tegmental area (VTA). Following the lesion, almost 40% of the animals in that study exhibited considerable reductions in the rewarding value of the stimulation. In addition, data from the same investigation suggested the existence of an interhemispheric component in the amygdaloid modulation of MFB reward signals - lesion effects on reward thresholds were observed in MFB structures ipsi - and contralateral to the lesion. Although we interpreted these data as evidence of an amygdaloid modulation - in particular of the cortical amygdaloid nuclei - of MFB reward signals, we were unable to identify a consistent relationship between lesion size and/or site and threshold change.

In light of these findings, the present investigation combined behavioural and metabolic techniques to pursue two major goals. The first was to identify with more accuracy which of the

amygdaloid sub-nuclei are involved in modulating MFB reward signals, hypothesizing that lesions to cortical aspects of the amygdala would alter thresholds for rewarding MFB stimulation. Such a result would be of paramount importance given the fact that lesions of extra MFB structures typically have little or no effect on MFB reward thresholds (see Simmons, Ackermann, and Gallistel, 1998).

Second, it was of particular interest to further explore the nature of the interhemispheric contribution to the amygdaloid modulation of MFB self-stimulation. In the rare instances that lesions are effective in altering MFB thresholds, (Arvanitogiannis et al, 1996; , Gallistel, Leon, and Waraczynski, 1996; Waraczynski, and Perkins, 2000), these changes are usually restricted to sites within the damaged hemisphere. We reasoned that if there is interhemispheric transmission of amygdaloid-MFB reward signals, as suggested by the data from our previous study (Bielajew, Miguelez, and Shiao, 2002), then we should witness threshold changes in both hemispheres following a single amygdaloid lesion. Furthermore, a more prominent functional damage of the amgydala (as assessed by cytochrome oxidase histochemistry) should presumably be correlated with greater modifications in the reward value of MFB stimulation. Finally, given our hypothesis that certain nuclei of the amygdala modulate reward signals from the LH and VTA, we suspected that lesions of these structures would have a metabolic impact on MFB sites supporting self-stimulation.

3. Materials and Methods

3.1 Subjects and surgery

The subjects were fifteen male Long-Evans rats (Charles River Canada, St-Constant, Québec, Canada) weighing 300 to 400 g at the time of surgery. All procedures were done in accordance with the guidelines of the Canadian Council of Animal Care. Deep anaesthesia was induced by an intraperitoneal injection of 65 mg/kg of sodium pentobarbital (Somnotol). Using standard stereotaxic techniques, each animal was prepared with four monopolar electrodes - one bilateral pair was aimed at the cortical nuclei of the amygdala and a second bilateral pair at the MFB to target either the LH or the VTA. These sites were chosen on the basis of the results from our previous investigation (Bielajew, Miguelez, and Shiao, 2002); amygdaloid lesions were shown to affect thresholds derived from both MFB placements. The stereotaxic coordinates, based on the Paxinos and Watson (1986) atlas, were as follows: Amygdala - 2.55 mm posterior to bregma, 3.8 to 4.1 mm lateral to the midsagittal suture, and 9.6 to 9.8 mm below the skull surface measured at bregma; MFB - from 3.0 mm to 4.8 mm posterior to bregma, 0.7 to 1.6 mm lateral to the midsagittal suture, and 8.3 to 8.4 mm below the skull surface measured at bregma. A stainless-steel wire, wrapped around four skull screws, served as the current return. A recovery period of one week followed surgery. During the study, the rats were individually housed in plastic cages and kept on a 12-h light/12-h dark cycle with light onset at 0700 h. Purina rat chow and water were available ad libitum. In order to control for lesion effects on metabolism and overall health status, the amount of food consumed as well as body weight were recorded every second day during the study. To standardize weight gain as a function of food ingested, the ratio of the two measures was calculated, usually referred to as percent efficiency of food utilization

(Bernadis, and Bellinger, 1979; Boyle, Storlien, and Keesey, 1978; Stenger, Fournier, and Bielajew, 1991).

3.2 Screening for self-stimulation, stabilization of thresholds, and electrolytic lesions

The rats were screened for self-stimulation at each of the MFB electrodes using conventional shaping procedures. The tests were conducted in operant chambers made of Plexiglas and wood (28.6 width x 32.6 length x 30.6 depth cm), in which a lever was located 3 cm above the floor. Stimulation was supplied by constant-current amplifiers (Mundle, 1980) and integrated circuit pulse generators (built in-house). It was activated by lever depressions, each of which produced a 0.5 s or 0.25 s train of monophasic cathodal rectangular pulses of 0.1 ms duration with an initial frequency of 40 Hz, and currents ranging from 250 to 400 μA across animals. In order to avoid the build-up of charge at the electrode/tissue interface, the current was shunted to ground between pulses via a low resistance pathway. Stimulation parameters were continuously monitored on an oscilloscope by reading the voltage drop across a 1 kΩ precision resistor in series with the rat. These procedures are described elsewhere (see Bielajew, Miguelez, and Shiao, 2002).

The inclusion criterion for MFB sites was a consistent response rate of more than 30 bar-presses per minute from at least one of the two MFB electrodes, and no obvious motoric or seizure activity. Once reliable responding was observed, frequency thresholds were determined from rate-frequency functions. These were based on a series of 60 s trials, during which the current was held constant, and the frequency of pulses reduced in 0.1 $\log_{10}$ steps each trial from a value that produced that animal's highest rate of responding to one that generated few or no bar-

presses. A sequence of four to five frequencies was usually necessary to observe this pattern. Thresholds were interpolated from the rate-frequency function and corresponded to the frequency value associated with half of the maximum response rate. For a more extensive discussion of the threshold methods, see Stellar and Stellar (1985). The curves were generated daily until the threshold values did not vary by more than 0.05 $\log_{10}$ units for three consecutive days. Our experience indicates that once stable according to this criterion, threshold values are usually maintained for months (Konkle, Bielajew, and Fouriezos, 2001).

Although thresholds were not determined at the amygdaloid sites, all behaviors stemming from stimulation of these loci, including bar-pressing, were recorded and classified according to the following scheme. Behaviors suggesting pain, fear, or discomfort, such as jumping backwards, shaking, freezing, piloerection, or rearing were defined as aversive, and behaviors such as exploration, sniffing, approach towards the lever, or bar-pressing were defined as rewarding. This procedure was done in order to examine the correspondence between behaviors stemming from amygdaloid stimulation and the impact of post-lesion effects on MFB frequency thresholds. Note that other than a few non-contingent priming trains of pulses, delivery of amygdaloid stimulation was contingent on bar-pressing behaviour and stimulation parameters matched those used for the ipsilateral MFB electrode of that animal in terms of train length, pulse duration, and lowest frequency. However, in order to prevent seizures and any potential aversive consequences, the initial current was set as 200 μA, and increased only until the effects of the stimulation could be readily assessed, according to the above definition.

Once MFB threshold values were considered stable according to the above criterion, rats were briefly anaesthetised with the inhalant Halothane and a unilateral electrolytic lesion (6.0

volts anodal current) applied for 20 s to the amygdaloid site ipsilateral to the MFB electrode associated with the lowest frequency threshold in the case of animals responding bilaterally for MFB stimulation. In animals with only one admissible MFB electrode (see inclusion criterion), the lesion was made to the amygdala on the same side. Following this procedure, MFB frequency thresholds were determined (ipsilateral to the lesion, and contralateral if appropriate) the next day and thereafter every second day for a total of twenty-one days.

At the end of this twenty-one day period, animals were once again anaesthetised with Halothane and a second amygdala lesion induced, this time on the contralateral side. Again, thresholds were collected from one (unilaterally implanted animals) or both (bilaterally implanted animals) MFB sites on the day following the second lesion, and subsequently on every second day for a total of twenty-one days. In summary, thresholds were determined at all MFB electrode sites for a total of forty-two days, twenty-one after the first lesion and twenty-one after the second one.

3.3 Histology

After completing all behavioural tests, animals were euthanised with an overdose of sodium pentobarbital (Somnotol). The eleven animals used for cytochrome oxidase analysis were perfused intracardially with an initial saline wash followed by a solution of 2.5 % paraformaldehyde and 4% sucrose in a 0.1 M phosphate buffer (pH 7.4), and a final wash of cold 0.1 M phosphate buffer (pH 7.4). The remaining four rats were perfused intracardially with an initial saline injection followed by a solution of 10% formalin. Brains processed for cytochrome oxidase histochemistry were stored in a 0.1 M phosphate buffer solution (pH 7.4) containing 10%

sucrose. The others were stored in 10% formalin and subsequently sectioned at a thickness of 30 μm and stained with Nissl in order to locate the lesions and electrode tips. Tissue retained for the histochemical analysis was likewise sectioned at 30 μm and prepared for cytochrome oxidase histochemistry, according to the protocol described by Wong-Riley (1979), except that the reaction was done on slide-mounted sections rather than on free-floating tissue. Of these brains, every alternate section was saved for routine Nissl staining.

3.4 Cytochrome oxidase histochemistry

Tissue sections were incubated for 45 min in 0.1 M phosphate buffer (pH 7.4) containing 0.027 mM of Cytochrome C (Sigma-Aldritch, Canada), 1.542 mM of diaminobenzidine (Sigma-Aldritch, Canada), and 131.45 mM of sucrose. The reaction was stopped by three rinses in 0.1 M phosphate buffer. Sections were mounted on slides, air-dried, and cover-slipped. Digitized images were obtained for densitometry readings using an Optronics Inc. system.

Mean relative optical densities (rod) were measured via a microcomputerized analysis system (Internet UTHSCSA Image Tool analysis, 2001) from the following MFB and amygdaloid sites: 1) around each amygdaloid lesion, 2) inside each amygdaloid lesion, and 3) around each MFB electrode. For each reading, background density based on a clear portion of the slide was subtracted and the difference in logarithmic values computed. For each animal and for each of the three regions defined above, rod values from ten different areas were collected and averaged to provide a single representative index, which was used in all analyses.

4. Results

4.1 Behavioral results

Of the 14 animals that achieved stable frequency thresholds for BSR, six responded on both electrodes and eight on one electrode. Thus, all analyses as well as figure presentations are based on 20 electrode sites, divided into three groups as follows. Group 1 comprises the bilaterally implanted animals (1323e1, 1416e1, 1417e1, 1421e1, 1423e1, and 1425e1, in which thresholds were collected from the MFB electrode ipsilateral to the lesion). The same animals belong to group 2, bilateral-contra, except that in this case thresholds were obtained from the electrode located contralateral to the first lesion (1323e2, 1416e2, 1417e2, 1421e2, 1423e2, and 1425e2). The suffixes e1 and e2 attached to animal numbers refer to the position of the electrode relative to the first lesion - e1 (ipsilateral to lesion 1), and e2 (contralateral to lesion 1). In addition to these two groups, thresholds from eight animals responding from one electrode only were tracked from the MFB site ipsilateral to the lesion (1327, 1329, 1418, 1419, 1427, 1430, 1431, and 1434). To facilitate comparisons between these data and the bilateral-ipsi animals, this Group was labelled "1a". Note that in these animals, the amygdaloid lesion was always applied to the same side as the functional MFB electrode, and that there was no separate unilateral-contra condition. Finally note that grouping designation was applied after the study was completed for purposes of data analyses and presentation.

Data are expressed in terms of percent change from baseline in frequency thresholds for clarity of data presentation. However, all statistical analyses (SPSS, 1998) were done on actual frequency thresholds and charge values, the latter to take into account the total amount of stimulation associated with each threshold determination. While pulse duration was held

constant across animals, current was not. Each threshold value was converted to total charge using the formula Q = IND, where Q is the charge in microcoulombs (μC), I = the current in microamperes (μA), N = the threshold determined for the number of pulses in the 500 or 250 ms stimulation train, and D, the pulse duration in seconds (0.0001) (Gallistel, 1978).

No significant differences were found among groups in baseline frequency thresholds, currents, or charge values. These data were respectively, for Group 1 (bilateral-ipsi): 43.23 ± 10.42 Hz, 298.33 ± 24.69 μA, and 0.58 ± 0.10 μC; for Group 1a (unilateral only): 29.33 ± 4.43 Hz, 276.25 ± 12.81 μA, and 0.42 ± 0.08 μC; and for Group 2 (bilateral-contra): 47.07 ± 10.55 Hz, 298.33 ± 24.69 μA, and 0.62 ± 0.11 μC. Note that in animals with functional bilateral implants, the thresholds generated at both MFB electrodes were associated with the same current value. In all animals, bar-pressing behavior was unaltered over the course of the experiment, with no motor or seizure-like activity induced as a result of repeated stimulation.

Although threshold determinations were not conducted at amygdaloid sites, each was screened for self-stimulation and all stimulation-induced behaviors were recorded before lesions were produced, and classified according to the criterion elaborated in the Methods section. This procedure was implemented in order to reveal any correspondence between loci categorized as rewarding or aversive and the magnitude of post-lesion effects on MFB frequency thresholds; this was not found to be the case. All correlations were weak and failed to reach significance. Note that amygdala stimulation did not generate any overt seizure activity. Moreover, lesions did not affect metabolism as assessed by the index of percent efficiency of food utilization (see Methods section). This measure was stable in all groups.

Because the analyses of charge and frequency threshold values yielded identical patterns,

we report only the results associated with the latter, more commonly used measure. Figure 17 shows the average frequency threshold, expressed as percent change from baseline, for the three groups of animals during the study. Twenty-four hours following the first amygdaloid lesion, average threshold values determined from the electrode contralateral to it (Group 2) were already elevated, approaching asymptotic levels by the third session or sixth day post-lesion, at which point thresholds were 150% of baseline values in one case, indicating a reduction in the rewarding value of the stimulation. A mixed ANOVA performed on the three groups confirmed this visual impression, revealing a significant effect of time ($F(2,24) = 1.781$; $p = 0.014$) and interaction between time and group ($F(2,48) = 1.430$; $p = 0.037$). Use of the conservative Greenhouse-Geisser correction formula increased both probabilities slightly above 0.05. In comparison to group 2, groups 1 and 1a were much less affected by either amygdaloid lesion, with thresholds remaining closer to baseline values throughout the study.

Following application of the amygdaloid lesions, we examined their impact on individual MFB reward thresholds. We defined any threshold deviation exceeding 0.10 $\log_{10}$ units (26%) from baseline frequency thresholds as reflecting a lesion effect on MFB reward, a relatively conservative criterion. Recall that prior to any damage, MFB-derived thresholds were considered to be stable if they did not vary by more than 0.05 $\log_{10}$ units for three consecutive days. Figure 18 shows the percent change from baseline frequency threshold for the six electrode placements exhibiting a post-lesion effect as defined above.

Two Group 2 placements (electrodes contralateral to the first amygdaloid lesion) (1417e2 and 1421e2) showed immediate and sizable post-lesion increases in frequency thresholds (decreases in the rewarding value of the stimulation). The peak threshold changes were observed

roughly seven days post-lesion and corresponded to a change from baseline values of 160%, (0.5 $\log_{10}$ units); they were thereafter maintained for the duration of the forty-two days study. Application of the second lesion did not generally alter this pattern. In other words, the threshold increase associated with the first lesion persisted after the second one was executed, perhaps due to the fact that threshold shifts had reached a ceiling. To our knowledge, these are by far the largest and most enduring lesion effects on MFB thresholds ever reported following damage to a contralateral structure. One animal's placement (1323e2) showed a decrease in frequency thresholds (increase in the rewarding value of the stimulation) of about 17% (0.07 $\log_{10}$ units) relative to its baseline threshold. This effect was immediately observed following the first lesion and increased to about 35% (0.14 $\log_{10}$ units) after the second one. The MFB frequency thresholds of three other placements in this group (1416e2, 1423e2, 1425e2) were unaffected by either amygdaloid lesion (data not shown).

Two Group 1 electrode placements (1421e1 and 1417e1) exhibited decreases in the rewarding value of the stimulation (increase in frequency thresholds) ranging from 30 to 40% (0.12 to 0.15 $\log_{10}$ units). This effect was evident five days after the first amygdaloid lesion and generally maintained after the second one. Recall that these MFB loci were located on the opposite side of those MFB sites showing the largest post-lesion threshold shifts. Thus, lesions were more effective in altering MFB thresholds contralateral to the amygdaloid damage than those ipsilateral to it. In Group 1, thresholds from four other electrode placements (not shown) were not influenced by either lesion (1416e1, 1423e1, 1323e1, and 1425e1). Finally, one Group 1a placement exhibited a gradual decrease in the rewarding value of the stimulation after the first lesion. It reached a maximum of 60% after damaging the amygdaloid complex contralateral to it,

possibly reflecting a delayed effect of the first lesion. Note that across animals, the first and second lesion effects on frequency thresholds in bilaterally implanted animals were highly correlated (Spearman's rho = 0.90; p = 0.04).

Figure 19 shows the maximum response rate over the course of the study for the three groups of MFB placements. A mixed ANOVA with three independent groups and 25 repeated sessions revealed no significant difference in maximum rates between groups over time, suggesting that group designation did not influence overall performance during any of the three phases of the study - baseline, post-lesion 1, and post-lesion 2.

Although the statistical analysis did not detect significant group differences in maximum rates (see Figure 3), we had to confirm that post-lesion threshold shifts in individual animals were not related to lesion-induced changes in performance or maximum rates, but to changes in the rewarding value of the stimulation per se. Consequently, we computed the correlation between frequency thresholds and the maximum rate data in each animal. All correlations were insignificant with the exception of placements 1431 ($r = -0.90$, $p = 0.00$), 1421e1 ($r = -0.79$, $p = 0.00$), and 1417e2 ($r = -0.84$, $p = 0.00$). For these three placements, a one-way repeated measures ANCOVA on threshold values with maximum rates as the running covariate was performed (Winer, Brown, and Michels, 1991). Partialling out the contribution of maximum rate did not remove the significant effect of time in each case; 1431: $F(24,48) = 9.11$; $p = .004$; 1421e1: $F(24,48) = 15.38$; $p = 0.02$; 1417e2: $F(24,48) = 81.17$; $p = .001$.

Figure 20 shows both amygdaloid lesion placements for the animals in the study, arranged in anteroposterior order. The annotations "L1" and "L2" at the bottom of each illustration indicate the location of the first and second lesions respectively. Note that due to histological

error, we were unable to determine the lesion placement associated with rat 1416. In Figure 21, electrode placements are depicted, again arranged according to anteroposterior location. The left column shows MFB sites for the bilateral group of animals and the right for the unilateral group. In both Figures 4 and 5, the letters "R" and "L" on top of the drawings indicate right and left placements respectively.

In all animals, the side of the first lesion (right or left) did not appear to be related to the magnitude of post-lesion MFB threshold shifts. Additionally, we looked at potential links between threshold change and lesion size and found none. However, damage which affected placements 1417 e1, 1417e2, 1421e1 and 1421 e2, corresponding to the largest threshold shifts, was located in anterior sites which also included cortical amygdaloid nuclei. A one-way ANOVA was performed to evaluate if there were differences in the anterior-posterior location of the lesions that corresponded to the presence or absence of post-lesion changes in thresholds. Animals exhibiting alterations greater than 0.1 $\log_{10}$ units from baseline had significantly more anterior lesion placements than rats showing no modification in reward (shifts less than 0.1 $\log_{10}$ units from baseline): $F(1, 23) = 8.37$; $p = 0.008$. Moreover, in the case of the former, lesions 1 and 2 were located at roughly the same anteroposterior level. The lesions of animal 1431 were misaligned on the ventral plane and only the second one encompassed some portions of the anterior cortical amygdaloid nuclei. Finally, amygdaloid damage that gave rise to the post-lesion MFB increase in reward (1323e2) touched a portion of the basomedial amygdaloid nucleus and obliterated almost two intercalated nuclei in the case of lesion 1; lesion 2 destroyed parts of the medial amygdaloid nucleus and a small portion of the anterior cortical nucleus.

4.2 Cytochrome oxidase results

Eleven of 15 animals were retained for this part of the study. Non-parametric Wilcoxon matched pairs tests were first conducted on density values to assess global differences between animals having two MFB electrode placements (n = 5) and those with only one functional electrode (n = 6). Recall that all calculations were based on a single representative density index for each region of interest, based on the average rod values taken from ten different areas within that target locus. The pairs of measures used to compute the Wilcoxon difference score were obtained from the same area of interest in animals responding for unilateral versus bilateral stimulation. Thus, our matching criterion was the target area under scrutiny. No significant differences were detected in reaction product density within and around each of the lesion sites. However, examination of the bilateral group alone revealed a significant density difference between the two lesions within ($T_5 = 0$; $p = 0.04$) and around ($T_5 = 4$; $p = 0.04$) their sites, indicating that in these animals, the first lesion contained a significantly higher amount of reaction product than the second one.

The same exercise applied to the evaluation of reaction product density around the tips of the MFB stimulating electrodes revealed no significant difference in the bilateral group, or between unilateral and bilateral groups. Likewise, no prominent differences were found in the size of the lesions across animals, done by comparing area measurements of lesion sites in individual animals.

For each electrode placement, a lesion index, based on the average percent change from baseline (in thresholds) across sessions, was computed and correlated with rod values obtained within each lesion. Recall that in correlations considering electrode 1 (ipsilateral to lesion 1),

both unilateral and bilateral animals were taken into account (n = 11). Conversely, only bilateral animals (n = 5) could provide data for correlations computed with electrode 2 (contralateral to lesion 1). A significant negative correlation suggests that a large threshold effect is associated with a lesser reaction product density in the analysed tissue while a high positive correlation indicates the reverse.

Across animals, a significant positive correlation was found between the size of the threshold shift in electrode 1 and the density of cytochrome oxidase reaction product within the lesion on the same side (lesion 1; $r = 0.62$, $p = 0.04$). However, threshold shifts from the same electrode were negatively correlated with the density of reaction product density inside the contralateral lesion (lesion 2; $r = -0.90$, $p = 0.04$). The importance of these data with respect to interhemispheric connectivity is discussed below. Other correlations failed to reach significance.

Figure 17. This figure illustrates the percent change from baseline frequency threshold for all MFB placements divided into the three following groups. Groups 1 and 2 comprise placements of animals with a bilateral pair of functional MFB electrodes, either ipsilateral (Group 1) or contralateral to the first lesion (Group 2). Group 1a comprises the animals with electrodes ipsilateral to the first amygdaloid lesion in animals with one functional MFB electrode.

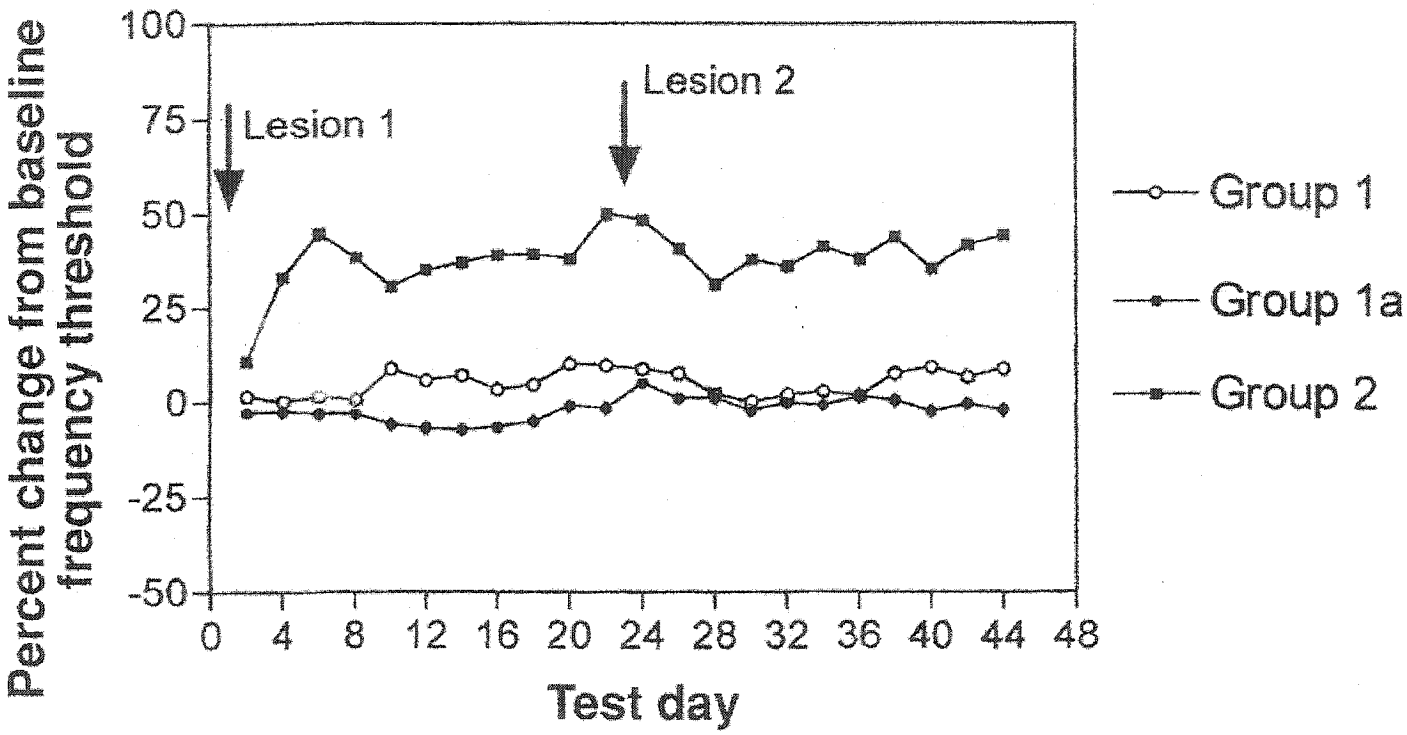


Figure 18. The percent change from baseline frequency thresholds is illustrated for all individual animals in which post-lesion threshold changes exceeded 0.10 $\log_{10}$ units.

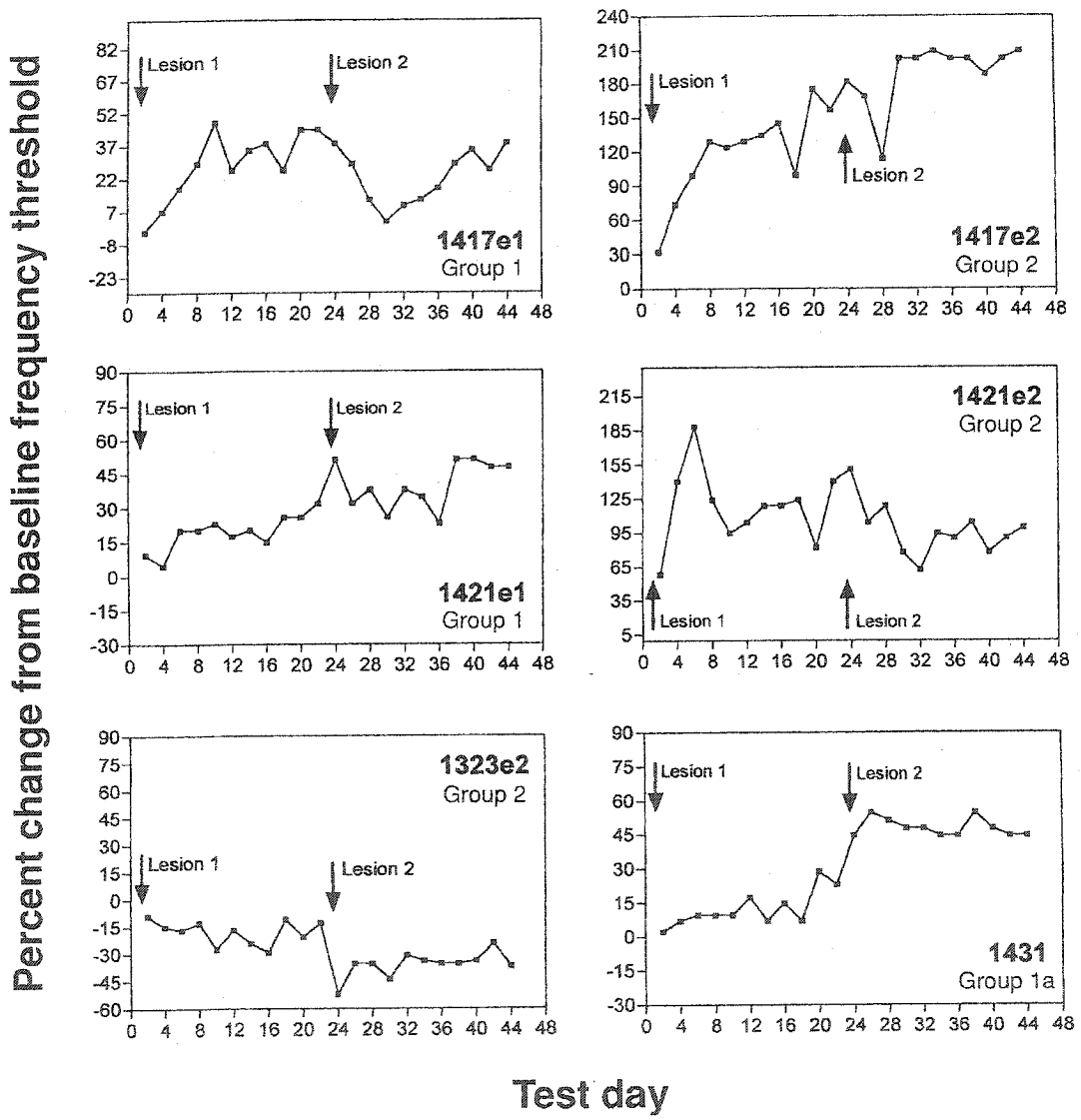


Figure 19. The figure shows the maximum response rates per minute that were obtained at all MFB sites. The data are divided into three groups as follows. Group 1a includes the eight placements ipsilateral to the first amygdaloid lesion associated with animals having one functional MFB electrode. Group 1 includes the six animals with placements ipsilateral and Group 2 contralateral to the first lesion. The first three sessions represent baseline maximum rates, and the rest, the rates obtained after the first and second lesions (indicated by the arrows).

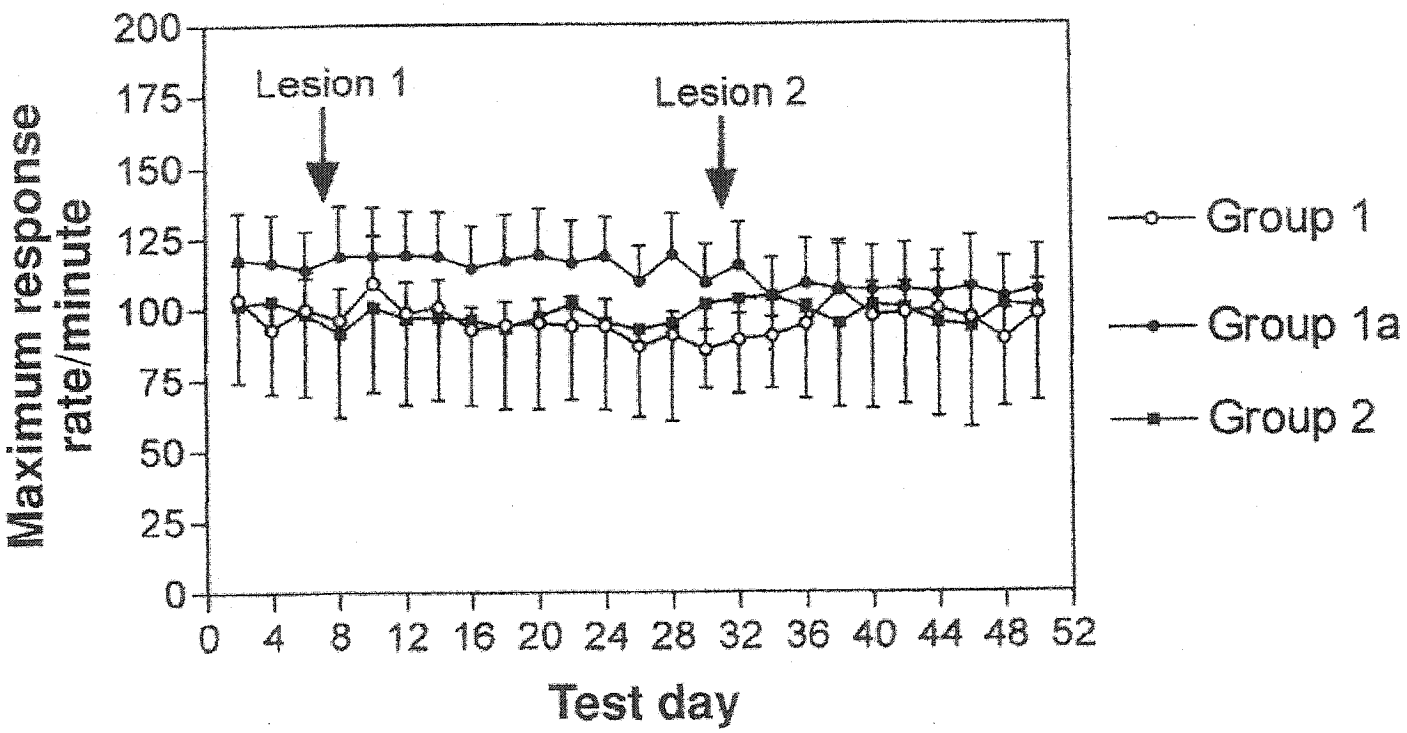


Figure 20. Tracings of anatomical figures adapted from the Paxinos and Watson (1998) atlas showing the location of both amygdaloid lesions in all animals. The tracings are ordered from top left to bottom right, beginning with the most anterior lesion site associated with the right amygdala. The letters "R" and "L" at the top of each column refer to the right and left hemispheres respectively. The rat identifier (R) is centered at the top of each pair of plates. The annotations "L1" and "L2" identify if the plate refers to the location of the first or second lesion.

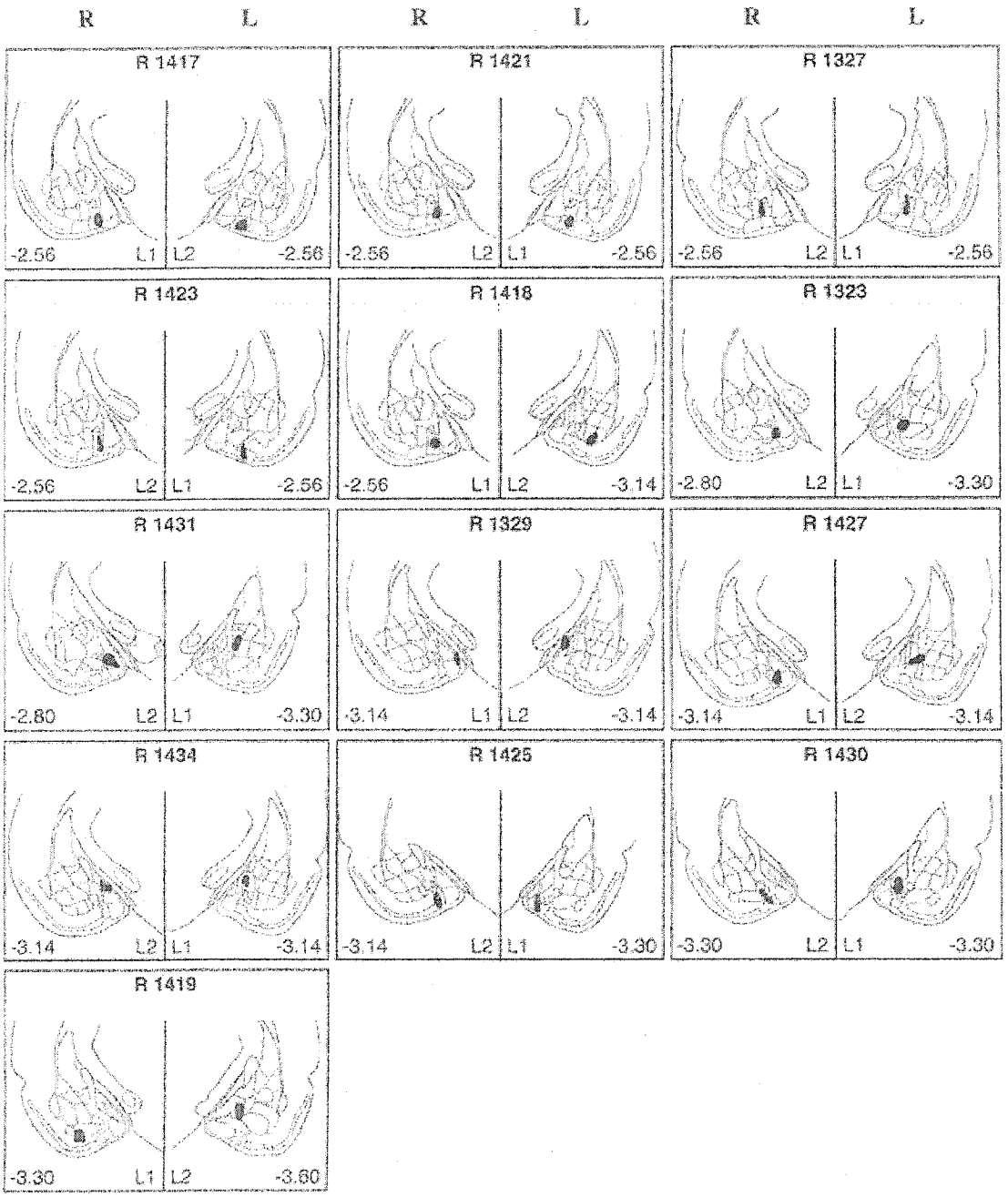
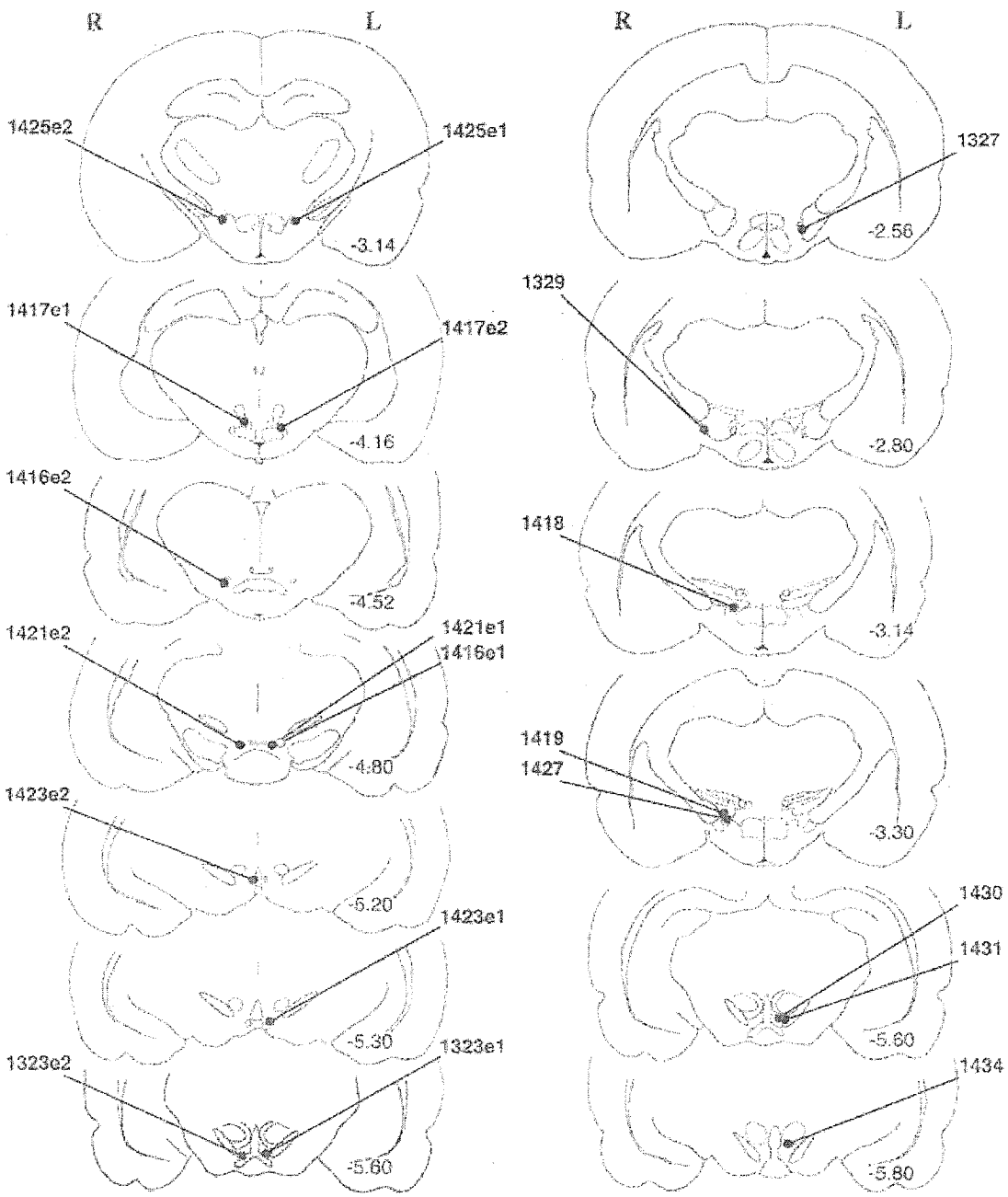


Figure 21. Tracings of anatomical figures adapted from the Paxinos and Watson (1998) atlas, showing the location of MFB placements (filled circles) for all animals. Drawings are arranged from top to bottom, beginning with the most anterior. The plate's location in millimetres relative to bregma is shown on the bottom right corner of each tracing. The four digit numbers identify the rat and the attached suffix if the site is ipsilateral (e1) or contralateral (e2) to the first lesion. Animals in the bilateral group (two functional MFB electrodes) are represented in the left column and animals in the unilateral group (single functional MFB electrode) in the right column. The letters "R" and "L" at the top of each column refer to the hemisphere side of implant.



5. Discussion

The use of lesion techniques as a method to gain insight into the circuitry underlying MFB reward has generally had disappointing outcomes. Seemingly comparable lesions, whether aimed at specific or extra-MFB structures typically have had variable effects on MFB thresholds (see Simmons, Ackerman, and Gallistel, 1998) regardless of lesion site or size. Moreover, post-lesion impacts on the rewarding value of MFB stimulation have been found to be usually modest, rarely exceeding shifts of 0.10 $\log_{10}$ units (26%) in comparison to baseline values (see, for example, Acheson et al., 2000; Gallistel et al., 1996; Waraczynski and Shizgal, 1996). Although large effects were observed in the present investigation, change in threshold was not related to lesion size. As in the case of our previous study (Bielajew, Miguelez, and Shiao, 2002), animals displaying post-lesion modifications in the rewarding value of the stimulation had lesions comparable in dimensions to those exhibiting no threshold alterations. A further consistency was that no differences were found between LH and VTA placements in terms of post-lesion effects on reward thresholds, suggesting that amygdaloid structures modulate signals from a continuum of MFB structures. This idea has anatomical validity on two fronts. First, it is known that although the majority of the projections from the amygdaloid complex to the MFB terminate in the LH, some fibers of the ventral amygdalofugal pathway extend to the VTA (Hopkins and Holstege, 1978; Prewitt and Herman, 1998; Van der Kooy et al., 1984). Second, evidence based on the results of double-pulse tests of connectivity indicates that some LH and VTA reward fibers are one and the same (Bielajew and Shizgal, 1982, 1986; Shizgal et al., 1980).

Although the literature concerning the amygdaloid complex with respect to MFB reward is scarce, the structure's ability to mediate BSR (Kane, Coulombe, and Miliaressis, 1991a;

Panagis et al., 1995; Touzani and Velley, 1998) is in itself an important indicator of its role in the processing of rewarding stimuli. Notwithstanding the fact that maximum rates of self-stimulation in the complex are commonly quite low, reliable thresholds can be obtained in many of its sub-regions (see Kane, Coulombe, and Miliaressis, 1991a). Moreover, it has been reported that contingent stimulation of various nuclei of the amygdaloid complex decreases thresholds for self-stimulation in the MFB (Szabo, Rozowska, and Kolta, 1972).

Other lines of evidence, however, suggest that amygdaloid and MFB reward signals do not converge onto a common neuroanatomical substrate. Kane, Coulombe, and Miliaressis (1991b), for instance, found that amygdaloid and MFB rewarding effects did not summate. Furthermore, Waraczynski et al. (1990) found no threshold alteration in the MFB stimulation following lesions of the amygdaloid complex. These apparent contradictions may be reconciled by the supposition that amygdaloid sub-regions play different roles in modulating MFB reward. In support of this conjecture, one early investigation (Jackson and Gardner, 1974) showed that stimulation of the basolateral amygdala had no impact on rates for self-stimulation from the LH, whereas stimulation of the corticomedial amygdaloid nucleus inhibited them.

In agreement with the idea that amygdaloid sub-regions do not modulate MFB reward to the same extent are the differences found when comparing lesion targets used in more recent investigations. For instance, Warazcynski's (1990) relatively ineffective lesions were located in the amygdalofugal pathway as well as in the medial and basolateral sub-nuclei. In contrast, damage which produced modifications in MFB reward in the present study encompassed other portions of the complex. Destruction of anterior sites encroaching on cortical amygdaloid nuclei appeared to be more efficient than lesions to other amygdaloid loci, some producing threshold

changes greater than 150% from baseline values. This pattern was also observed in our previous investigation (Bielajew, Miguelez, and Shiao, 2002), in which four of the five most effective amygdaloid lesions also damaged cortical amygdaloid nuclei located roughly at the level at which the magnocellular nucleus of the lateral hypothalamus appears (Paxinos and Watson, 1986). As in the case of this study, lesion effects on thresholds were uncommonly large and sustained.

Although such results testify to the importance of the cortical amygdaloid nuclei in modulating MFB reward, many aspects of this relationship still remain to be clarified. Similar to our previous data (Bielajew, Miguelez, and Shiao, 2002), lesion impacts were not consistent in direction, with one placement (1323e2) exhibiting an increase in the rewarding value of the stimulation. Interestingly, one feature distinguishing this animal's first lesion from our other ones was the obliteration of two intercalated nuclei. These clusters of GABAergic neurons (Pare and Smith, 1993) have been found to be particularly prominent in the rat amygdaloid complex (Price, Russhchen, and Amaral, 1987). The unique configuration of lesions encompassing a variety of amygdaloid nuclei, including the intercalated masses, may provide an explanation for the occurrence of the small threshold decreases found in the present study.

This investigation is the third in a series of behavioral and/or metabolic explorations carried out by our group (Bielajew, Miguelez, and Shiao, 2002; Miguelez et al., 2001) aimed at evaluating the contribution of the amygdaloid complex in the circuitry underlying MFB reward. These data all converge in suggesting the existence of a crucial role for the cortical nuclei of the amygdaloid complex in modulating MFB reward. Given these results, it is surprising that these sub-structures generally receive little attention. Data from early studies associate activity in this region with motivation for eating (Lukaszewska et al., 1984; Montgomery and Singer, 1975), as

well as with maternal and female sexual behaviour (Ellendorff and Parvizi, 1981; Warembourg, 1981) in laboratory rats.

In the general context of reward, much more attention has been given to other loci of the amygdaloid complex, such as the basolateral group. Setlow and his collaborators (2002), for instance, used *N*-methyl-aspartic acid to simultaneously lesion the basolateral amygdala and the contralateral nucleus accumbens of rats. In comparison with sham-lesioned and ipsilaterally damaged controls, the animals did not acquire second-order conditioned responses in a rewarding Pavlovian conditioning paradigm. Such associations are believed to be dependent on a first-order conditioned stimulus having achieved an appetitive value via its pairing with food (Setlow, Gallgher, and Holland, 2002).

In light of such data, one explanation for our findings is that MFB thresholds were affected not by destruction of the cortical amygdaloid nuclei per se, but rather by indirect damage to the basolateral area. Supporting this idea is the fact that boundaries between the two groups of nuclei are not easily distinguishable; extensive dendritic overlap exists in the region where the cortical nuclei merge with the basolateral ones to such an extent that the former appear to form an anatomical continuum with the later (McDonald, 1992).

Our findings suggest that the cortical amygdaloid nuclei are pivotal structures in the modulation of MFB reward. We showed that damage to rewarding sites in the LH affect long-term metabolic activity of the cortical amygdaloid nuclei as measured by cytochrome oxidase (Miguelez et al., 2001). Such an idea has anatomical validity. The anterior cortical nucleus has been shown to send efferents directly to the hypothalamus (Petrovich, Canteras, and Swanson, 2001), and both it and the posterior cortical nucleus project to the bed nucleus of the stria

terminalis (Price, Russchen, and Amaral, 1987). The latter also sends heavy projections to the hypothalamus so that this pathway is a likely candidate for a di-synaptic circuit through which the amygdala could modulate MFB reward (Price, Russchen, and Amaral, 1987). Moreover, the bed nucleus of the stria terminalis is part of a region called "the extended amygdala" (Johnston, 1923) also comprising the central amygdaloid nucleus and the dorsal substantia inominata (Koob, 1999). This area has been shown to be involved in the development of drug reward and addiction (Koob, 1998; Koob and Le Moal, 2001) and is thought to be a sensory-reward association area (Cassell and Freedman, 1999).

Another major pathway linking the amygdaloid complex with the MFB is the ventral amygdalofugal pathway. Like the stria terminalis, this fiber bundle arises from all amygdaloid nuclei with the exception of the lateral one (Krettek and Price, 1978; Rushchen and Price, 1984). It projects to the hypothalamus with some fibers ending in the VTA (Han and Ju, 1990). Interestingly, as early as 1963, Powell and his collaborators viewed this projection as a laterally directed extension of the MFB, with the stria terminalis representing a dorsal component of this bundle, separated from it by the internal capsule. Our lesion impacts on MFB reward may thus result from the inactivation of fibers exiting the cortical nuclei to merge either with the stria terminalis or with the ventral amygdalofugal pathway at rostral levels.

Another pivotal result from this study is that contralateral lesions were more effective in producing substantial reductions in MFB reward than ipsilateral ones, to our knowledge also unprecedented in studies investigating the circuit underlying MFB reward. Placements in which only a small decrease in reward was seen after lesions to the ipsilateral amygdala exhibited substantial ones following application of contralateral damage. This finding was supported by

our metabolic data. There were differences in the results of the correlational analyses between lesion 1 (applied first) and MFB threshold changes on both the ipsi and contralateral MFB sites. Recall, however, that this lesion was produced forty-two days prior to analysis, whereas the second one was produced only twenty-one days before histochemical processing. One possibility is that processes of regeneration occurring at the first lesion site over this month and a half period obscured potential links with MFB threshold changes. In this regard, it is important to note that the emergence of glia within lesion sites should have affected labelling only minimally since these cells have been shown to depend more on anaerobic glycosis rather than on the oxidative pathway as a means to derive their energy (Robinson et al., 1998; Wong-Riley, 1989).

On the other hand, cytochrome oxidase density within the second lesion was significantly and negatively correlated with threshold changes in the contralateral MFB. In other words, less reaction product density (signifying more destruction in the amygdala) was related to higher post-lesion effects on MFB thresholds, suggesting a functional role for these amygdaloid fibers in the circuit underlying MFB reward. Finally, the correlation between rod values within lesion 2 and threshold changes in the electrode ipsilateral to it was negative but failed to reach significance, possibly due to the smaller sample size in this group.

Although correlational data should be interpreted with caution, the correspondence between these analyses and our behavioral results strongly argue in favour of a functional interhemispheric role for amygdaloid modulation of MFB reward signals. One possible scenario is that this communication is accomplished by fibers that bifurcate at the level of the amygdaloid complex before sending projections to the MFB. All substantial effects on MFB thresholds were seen in animals with two functional MFB electrodes. In those cases, the first lesion produced

massive decreases in reward in the contralateral electrode but not in the ipsilateral one. Application of the second lesion, however, failed to modify thresholds in the electrode contralateral to it. One explanation for this is that the first amygdaloid lesion blocked signals originating from the contralateral amygdala, thus preventing communication between amygdaloid and MFB sites located in opposite hemispheres. The data from animals with only one functional electrode support this idea. These rats invariably failed to show substantial threshold changes following the second lesion.

The idea that the cortical amygdaloid nuclei share functional interhemispheric connections with respect to MFB reward has anatomical legitimacy. Dong, Petrovich, and Swanson (2001) showed that the posteromedial nucleus projects massively to its contralateral homologue through the contralateral bed nucleus of the stria terminalis. Recall that the latter is heavily connected to the MFB on the ipsilateral side, potentially conveying amygdaloid signals to it. Similar to the present case, earlier data from our laboratory (Bielajew, Miguelez, and Shiao, 2002) showed that lesions to cortical amygdaloid nuclei affected thresholds for contralateral MFB reward, although to a lesser extent than in this investigation. Waraczynski's (2003) findings also support the contention that the amygdala and the MFB communicate bilaterally with relation to reward: she found that lidocaine inactivation of target amygdaloid sites impaired BSR in the contralateral MFB.

Many more investigations need to be conducted to fully unveil the intricacies underlying amygdaloid modulation of rewarding MFB signals. However, taken together, our data indicate that the most rostrally located portions of the amygdaloid cortical nuclei are significantly involved in modulating MFB reward. Lesions aimed specifically at these sites generally produce

significant decreases in the rewarding value of MFB stimulation, suggesting that when intact, they play a role in augmenting the strength of MFB reward signals. Furthermore, amygdaloid damage alters thresholds both from ipsilateral and contralateral MFB sites, suggesting the existence of an interhemispheric communication pathway between these structures with relation to reward.

GENERAL DISCUSSION

Overview

The purpose of the investigations reported in this thesis was to explore the contribution of the amygdaloid complex in modulating rewarding brain stimulation elicited from the MFB. We designed the initial experiment with two postulates in mind. First, considering the electrophysiological, behavioural, and metabolic results with respect to these interactions obtained by others), it appeared very likely that the amygdaloid complex is involved to some degree in modulating MFB-derived rewarding brain stimulation. Second, data from previous investigations suggested that distinct sub-nuclei of this complex differentially influence a variety of behaviours, including reward. Considering the anatomical sophistication of the amygdaloid complex and its various components, it seemed probable that specific sub-nuclei were responsible for modulating reward signals elicited from MFB-derived self-stimulation.

Because the anatomical links between the amygdaloid complex and the MFB are known to be more direct with the hypothalamus than with any other MFB region, the first experiment was chiefly concerned with mapping the functional links between the LH (a noteworthy BSR site) and the amygdaloid complex through the use of a metabolic marker. Although a few investigations had examined metabolic changes in amygdaloid structures affected by BSR derived from this bundle, such studies were aimed at detecting immediate metabolic alterations through the use of such markers as 2-DG (Porrino et al., 1984, 1990) and c-fos (Arvanitogiannis, Waraczynski, and Shizgal, 1996; Hunt and McGregor, 1998; Nakahara et al., 1999). To our knowledge, tracking long-term amygdaloid metabolic activity in response to MFB-derived self-

stimulation had not yet been attempted. Thus, we chose to use the cytochrome oxidase marker, which reflects long-term metabolic activity rather than that concerned with more immediate demands (DiRocco, Kageyama and Wong-Riley, 1989; Erecinska and Silver, 1989). Our goal was to identify the specific amygdaloid sub-structure(s) that showed a modification of long-term neuronal activity following lesions to rewarding sites in the LH.

In our first study (Miguelez et al., 2001), we found that electrolytic lesions of rewarding LH sites significantly reduced oxidative metabolism, principally in the cortical amygdaloid nuclei, and to a lesser extent in the piriform cortex, thus suggesting their involvement in the modulation of BSR derived from this site. This evidence, however, was based not on behavioural, but strictly on metabolic data. We could not, therefore, be completely certain that the observed changes in metabolic activity reflected an amygdaloid modulation of self-stimulation *per se* since the effectiveness of lesions in reducing or eliminating BSR in the LH was not verified. Thus, we had to consider the possibility that our results reflected damage to MFB projections to cortical sub-nuclei of the amygdala that are only coincidentally related to BSR.

The second experiment (Bielajew, Miguelez, and Shiao, 2002) was designed to test the functional relationship between the amygdaloid cortical nuclei and the MFB with relation to reward. Additionally, we also wished to extend our exploration of this link to the VTA. This decision was motivated on the following grounds. First, anatomical data suggests that amygdaloid contributions to MFB reward may influence not only LH, but also VTA signals. Indeed, we knew that although the majority of the projections from the amygdaloid complex to the MFB terminate in the LH, some fibers of the ventral amygdalofugal pathway extend all the

way to the VTA (Hopkins and Holstege, 1978; Prewitt and Herman, 1998; Van der Kooy et al., 1984). Second, we were aware of direct psychophysical evidence showing that some LH and VTA reward fibers are one and the same (Bielajew and Shizgal, 1980, 1982, 1986; Shizgal et al., 1980).

Furthermore, in this experiment, we added yet another variable of interest, in that we examined whether the modulation of MFB reward by cortical amygdaloid nuclei extended to contralateral homologous MFB loci that supported BSR. Anatomical studies have shown that interhemispheric communication between the amygdaloid complexes are achieved through the anterior commissure. The posterior cortical nucleus has been found to project homotopically to the other hemisphere (Nitecka, Amerski and Narkiewicz, 1981), and also to the contralateral medial nucleus (Otterson, 1982). Before crossing in the anterior commissure, these projections course through the ipsilateral stria terminalis (Price, Russchen and Amaral, 1987) and appear to make synaptic connections in the bed nucleus of the anterior commissure and contralateral stria terminalis, suggesting the existence of secondary projections to other contralateral structures such as the hypothalamus (Dauth, Dafny and Gilman, 1976). Thus, in this part of the study, we were interested in exploring if amygdaloid modulation of MFB reward signals encompassed MFB sites contralateral to the amygdaloid lesion. Following these considerations, in the second experiment, we produced lesions in or around the cortical amygdaloid nuclei and evaluated the effects on a continuum of ipsi and contralateral MFB structures, from the LH to the VTA. Supposing that, as suggested by our first study, the cortical amygdaloid nuclei are indeed involved in modulating MFB reward, we expected lesions of these nuclei to alter thresholds for MFB self-stimulation.

In our second investigation (Bielajew, Miguelez, and Shiao, 2002) one group of animals

displayed post-lesion threshold increases up to 225%, among the most considerable reported to our knowledge. This result was in agreement with our hypothesis that specific amygdaloid sub-nuclei modulate MFB reward signals. However, small threshold shifts were observed in a second group, and a third one did not exhibit any post-lesion change in the rewarding value of the stimulation. Interestingly, the degree of post-lesion change appeared to be related to the location of the damage in that rats that showed substantial threshold modifications appeared to generally have a higher concentration of anterior lesions of the cortical and adjacent amygdaloid nuclei as compared to animals in the two other groups. This observation suggested that not only the specificity of the injured amygdaloid sub-nucleus, but also the anterior-posterior location of the lesion within these nuclei may be an important factor in determining the impact of amygdaloid influences on MFB self-stimulation. It may be that specific amygdaloid sub-nuclei located at more anterior sites modulate MFB reward signals through a diffuse collateralized organization of fibers.

Two other findings of the second study merited close scrutiny. The first was the fact that post-lesion effects were present not only in rats having electrode placements close to the LH, but also in those having VTA stimulation sites, thus confirming our hypothesis that amygdaloid modulation of MFB reward extends to this latter locus of reward. The second novel finding was the observation that threshold changes in the contralateral electrode echoed ipsilateral ones, suggesting the existence of interhemispheric links in the modulation of MFB reward by the amygdaloid complex. However, the nature of these links could not be clearly extrapolated from these data.

In light of these findings, the third experiment of this thesis (Miguelez et al., submitted)

had two major aims. The first objective was to further refine the levels (anterior-posterior) and target amygdaloid nuclei involved in the modulation of MFB reward signals. For this purpose, part of the study was designed in the same way as the previous one, in that we tested the impact of amygdaloid lesions on thresholds for LH and VTA self-stimulation. Additionally, we included some histochemical analyses using the cytochrome oxidase marker. Our aim was to examine if lesions of the cortical and adjacent amygdaloid nuclei would have a metabolic impact on VTA and LH rewarding sites. Recall that in the first study, we had used the histochemical technique to pinpoint the amygdaloid sub-nuclei which were metabolically activated in response to lesions to rewarding LH sites. Second, we wished to further explore the contribution as well as the nature of interhemispheric links in amygdaloid modulation of MFB self-stimulation. Thus, we examined the impact of serial bilateral amygdaloid lesions in animals having either unilateral or bilateral MFB electrodes that supported self-stimulation.

Here, we again obtained large and sustained post-lesion changes in MFB reward from a subset of animals. Similar to our last study, damage to anterior sites encroaching on the cortical amygdaloid nuclei was generally more effective in producing alterations in the reward value of the stimulation than lesions in other amygdaloid sites. The existence of interhemispheric connectivity between the MFB and the amygdaloid complex in the context of reward was corroborated both behaviourally and metabolically - contralateral lesions altered MFB reward to a greater extent than ipsilateral ones. Moreover, the density of cytochrome oxidase within amygdaloid lesions was negatively correlated with the increases in thresholds obtained at contralateral MFB sites.

Contribution of specific amygdaloid sub-nuclei in the modulation of MFB reward

The data presented in this thesis appear to globally converge in suggesting the importance of the cortical amygdaloid nuclei, more specifically anterior ones, in modulating MFB reward. In the first experiment, the largest metabolic activation occurred in the anterior cortical and posterolateral cortical sub-nuclei, respectively corresponding to the following levels: 1) the level at which the nucleus of the lateral olfactory tract is replaced by its bed nucleus (section -1.80 according to the atlas of Paxinos and Watson, 1998); 2) that portion of the piriform cortex just before its transition point (section -2.56 according to the atlas of Paxinos and Watson, 1998). In the second investigation, eight out of the ten effective lesions were located at or before the level at which the posterior portion of the basomedial amygdaloid nucleus separates from the posterolateral cortical amygdaloid nucleus (section -2.80 according to the atlas of Paxinos and Watson, 1998). In the final study, the most influential damage was situated at the level corresponding to that portion of the piriform cortex just before its transition point (section -2.56 according to the atlas of Paxinos and Watson, 1998).

The emergence of other trends found in our two behavioural studies, however, must be addressed. An obvious problem is the fact that the rewarding value of MFB stimulation in some animals having received a lesion appearing to encompass the most anterior cortical nuclei was unaffected. A few examples are animals 70, 80, and 95 from the second study and rat 1423 from the third one. Although in the first instance, effective lesions tended to be more medially located than ineffective ones, this was not found to be the case in the last investigation. On these grounds, the suggested hypothesis that the most influential amygdaloid modulators of MFB reward are the cortical nuclei which are located within the medial portions of the complex

appears improbable.

Another possibility is that our MFB threshold shifts were due not to the destruction of the cortical nuclei *per se*, but rather to damage produced in other amygdaloid structures. Such effects could, for instance, have been caused via processes of axonal degeneration and/or of damage to fibers of passage resulting from our electrolytic lesions. Moreover, in both behavioural studies, some effective lesions admittedly encroached on portions of the basolateral amygdaloid nuclei and some others on intercalated amygdaloid nuclei. The potential contribution of the latter was discussed earlier; they send heavy projections to the central amygdaloid nucleus (Collins and Paré, 1999) and have been shown to play a unique role in modulating a variety of motivated behaviours including reward (Royer and Paré, 2002; Royer, Martina and Paré, 2000). With regards to the basolateral division of the amygdaloid complex, anatomical data preferentially support the notion that central and/or basolateral amygdaloid structures rather than cortical ones are involved in the modulation of MFB reward. These nuclei are known to project significantly to the LH and to share intrinsic connections with the cortical sub-nuclei (Price, Russchen, and Amaral, 1987). Interestingly, recent behavioural data from Waraczynski (2003) suggest that the rewarding value of MFB stimulation can be significantly altered following temporary inactivation of the central extended amygdaloid complex.

The metabolic results obtained in the first investigation, however, strongly point to the cortical nuclei of the amygdaloid complex as significant structures in modulating MFB reward. Thus, even if we accept the interpretation that both the intercalated nuclei and portions of the extended amygdala are involved in such a modulation, this does not preclude, in our view, a potential role for the cortical amygdaloid nuclei as well. Furthermore, our different threshold

effects may be a consequence of secondary damage resulting from axonal degeneration and/or damage to fibers of passage occurring upon damaging the amygdala. In that regard, in may for instance be that some portions of the amygdala are involved in inhibiting the intensity of reward signals from the MFB whereas other loci would have the opposite effect. In such a scenario, lesioning an "inhibitory site" could have the effect of augmenting the reward value of the stimulation, while damaging an "excitatory" one could decrease it. It also follows from this model that a lesion that simultaneously encroaches on antagonistic systems would result either in small effects, depending on the extent of the damage to either system, or in no effect at all on reward thresholds.

This idea finds support from many lines of evidence. In a classic study of Wurtz and Olds (1963), for instance, whereas BSR was reported in the medial, central and cortical nuclei of the amygdaloid complex, stimulation-induced escape was elicited from stimulating the lateral and basolateral nuclei. In a similar vein, Jackson and Gardner (1974) they found that stimulation of the basolateral amygdala did not affect LH rates of self-stimulation which were inhibited by activation of the corticomedial nucleus.

More evidence pointing towards the idea that amygdaloid loci play different roles in modulating reward processes is provided by Fonberg and her collaborators (Fonberg, 1963, 1967, 1969, 1972; Fonberg and Delgado, 1961, Fonberg and Sychowa, 1968). For example, in some studies, dogs were shown to display surprisingly opposite behaviours following the application of serial electrolytic lesions to different parts of the amygdala. Damage produced to the central and medial nuclei resulted in aphagia, apathy, loss of interest in the environment, and in a general attitude which was described by the authors as "loss of the joy of life". When subsequently

injuring the lateral amygdaloid nuclei of the same dogs, these reactions were entirely reversed; animals again showed interest in food, and became as playful and friendly as they had initially been. The fact that two brain lesions resulted in behaviours similar to those displayed before any damage was provoked is in agreement with the interpretation of homeostasis, or, in other words, of a balance between excitatory and inhibitory influences of amygdaloid structures in reward.

More recent data lend further credence to this view. Watanabe et al. (2002) tested the impact of bilateral excitotoxic lesions of the central or basolateral nucleus of the amygdaloid complex on conditioned place aversion induced by morphine withdrawal. They found that whereas damaging the former attenuated withdrawal symptom in rats, injuring the latter had no impact on behaviour. These results were interpreted in terms of a differential involvement from the two sub-nuclei in the adverse affective components of morphine abstinence.

Ahn and Phillips (2002) also found functional differences between the central and basolateral amygdaloid nuclei, in this case in the context of food reward. They compared the effects of temporary inactivation of either the central or the lateral amygdaloid nucleus on dopaminergic efflux in the nucleus accumbens and medial prefrontal cortex. The experiment was carried out in food-deprived rats exposed to a palatable treat. Whereas inactivating the basolateral nucleus had no effect on dopamine levels and feeding behaviour, lidocaine infusions in the central nucleus attenuated dopamine release in both the nucleus accumbens and the medial prefrontal cortex. Interestingly, these changes were related to the anticipation and the consumption of the treat. The data were interpreted as indicating that the central but not basolateral amygdaloid nucleus interacts with the mesocorticolimbic dopamine system in providing a reward value to food.

Even at a molecular level, distinctions have been found to exist between subdivisions of the dorsal component of the lateral amygdala in response to aversive learning paradigms (Radwanska et al., 2002). The expression pattern of both extracellular signal-regulated kinases and a c-Fos transcription factor were mapped immunocytochemically. Following exposure to fear conditioning and avoidance training protocols in rats, extracellular-related kinases were shown to undergo phosphorylation within ventral but not dorsal aspects of the lateral nucleus. Similar results were obtained with c-Fos expression.

It should be noted that most of the existing research examining the role of the amygdala in reward-related processes has focused on lateral and/or on central amygdaloid structures whereas our data specifically point towards the involvement of the cortical nuclei in MFB stimulation. However, all the reviewed lines of evidence converge in suggesting that the sub-nuclei of this complex are differentially involved in motivation and reward.

In summary, it is conceivable that some of our lesions injured different portions of the complex, either by directly encroaching on them or by inducing a process of neuronal degeneration. Presumably, this could have resulted in the different patterns and directions of effects observed in our studies. What is further attractive about this idea is that it can also account for the delay in threshold modification observed in some of our animals, as discussed in the second paper reported here.

It is important to note that inconsistencies in terms of time-course, direction, and strength of effects on reward thresholds have traditionally been a trademark of studies using lesions as a mapping strategy. This occurs regardless of the injured locus, the lesion technique utilized, or the reward site under scrutiny. This fact is exemplified in Appendix 2, in which we provide a

detailed analysis of the data from some key studies in the lesion literature. Perhaps this is an indication that the functional specificity of amygdaloid sub-nuclei with respect to reward modulation is not unique to this structure and extends to other portions of the brain. Nonetheless, the use of lesions as a strategy to study functional anatomy is defendable on the grounds that significant and long-term threshold alterations unequivocally point at functionally linked lesion and stimulation loci. It should be emphasized that not only is this the case in these experiments, but also that such large alterations in the rewarding value of stimulation as we have found have rarely been reported (see Appendix 2).

Interhemipheric communication between the amygdaloid complex and the MFB with relation to reward

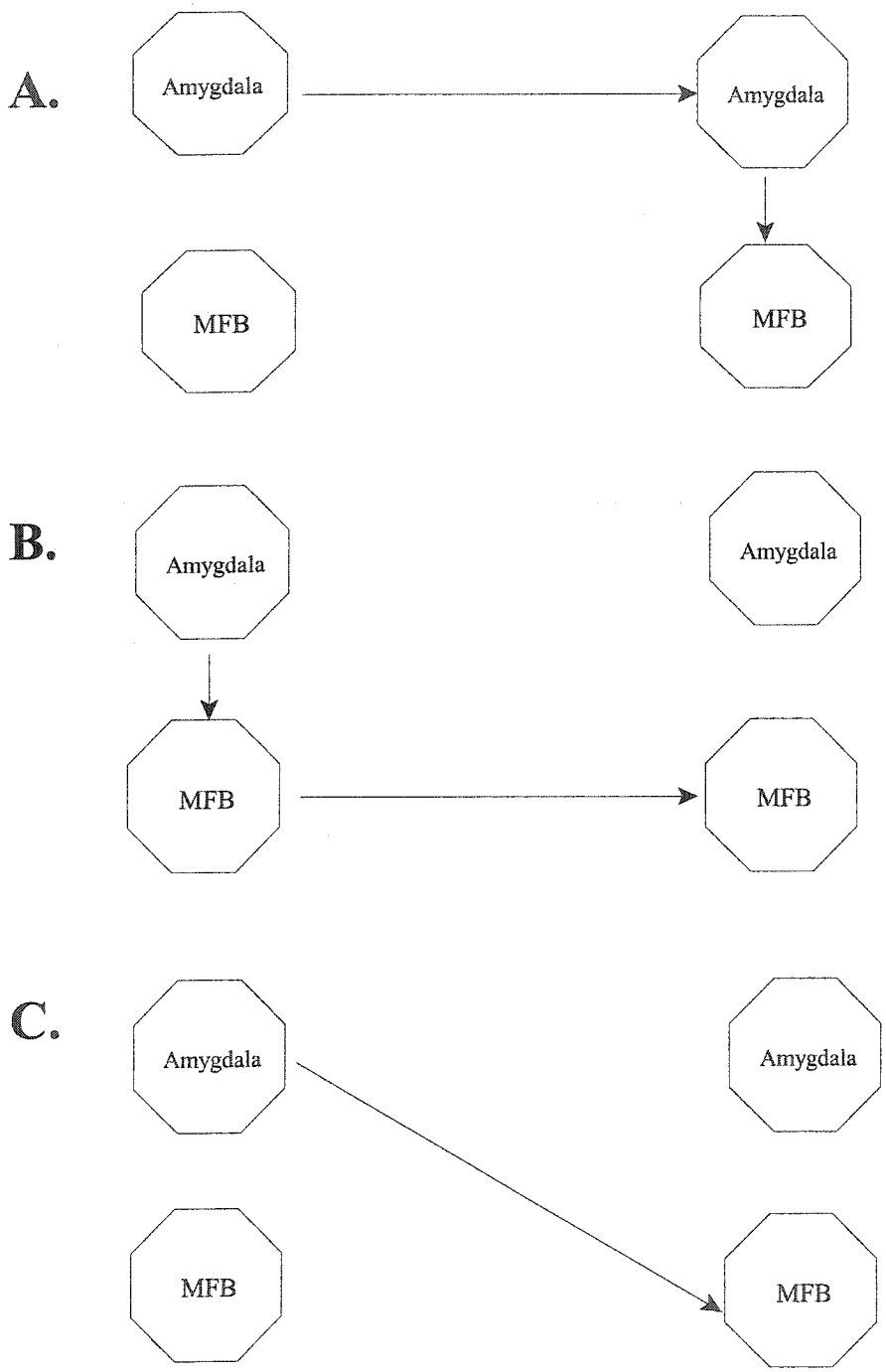
The other major finding that emerges from the studies presented in this thesis is that of the existence of an interhemispheric communication between amygdaloid and MFB loci with relation to reward. In our two behavioural investigations, effective lesions always modified the rewarding value of stimulation in both ipsilateral and contralateral MFB sites. Moreover, the results of the accompanying metabolic analyses in our last experiment revealed a strong negative correlation between the density of reaction product at damaged amygdala sites and threshold increases obtained at contralateral MFB loci.

Both our behavioural and metabolic data are indicative of bilateral cross-talk between amygdaloid and MFB structures with respect to reward. Three simplified routes of communication could potentially link these two structures, schematically illustrated in Figure 22. A number of observations from our last thesis experiment argue preferentially in favour of the

scenario depicted in Panel A of Figure 22 (amygdala to contralateral amygdala to ipsilateral MFB). The greatest threshold alterations were seen in animals with not one but two functional MFB electrodes. Applying the second lesion in these rats did not affect thresholds in the electrode contralateral to it, leading us to speculate that the effects of damage to the second amygdala could not be experienced due to the destruction of the first amygdala. Moreover, this hypothesis appeared to find corroboration from the fact that animals with only one functional electrode never showed substantial post-lesion threshold shifts.

The data from our second thesis study, however, are not consistent with this argument. Two of our "substantial" post-lesion threshold changes were seen in animals with only one functional electrode. Admittedly, it could be that had we been able to test the MFB placement contralateral to the lesion, threshold shifts may have been even greater than the ones observed on the ipsilateral side. However, this is not likely to have been the case since in bilaterally tested rats, changes in reward on the side contralateral to the lesion were of a similar or of a lesser magnitude than that on the ipsilateral side. The only explanation we can offer that can account for this puzzling discrepancy is one of a sampling nature. Given the small number of animals in our behavioural studies, it is difficult to know if our results are representative of different existing anatomical configurations. In other words, it is possible that not only one but two or even all of the suggested routes of communication presented in Figure 22 take part in the modulation exerted by amygdaloid structures on MFB reward. In this case, contralateral effects could vary depending on the affected system.

Figure 22. Hypothetical routes of interhemispheric communication between the amygdaloid complex and the MFB.



Anatomical as well as behavioural data support the existence of all proposed pathways of communication. Projections linking both amygdalas have been unveiled in the rat brain. They course through the stria terminalis before crossing over in the anterior commissure (Price et al., 1982), a scenario reminiscent of the route illustrated in Panel C in Figure 22. More recently, Dong, Petrovich and Swanson (2001) showed that the posteromedial cortical nucleus of the amygdala sends a substantial projection to the contralateral bed nucleus of the stria terminalis, a scheme theoretically situated somewhere between Panels B and C of Figure 22. Finally, although surprisingly little work has been conducted on crossed MFB connections, a number of behavioural investigations suggest the existence of such a pathway (see Stellar and Stellar, 1985).

One such line of evidence comes from bilateral stimulation data. As a control procedure in their collision experiment, Shizgal and his colleagues (1980) delivered pairs of pulses through bilaterally implanted LH electrodes. Although no inference of anatomical linkage could be made interhemispherically, the effectiveness of the pulse administered through the contralateral electrode ranged from 25 to 80%, indicating the presence of bilateral summation in the reward value of the stimulation. Also in agreement with the view that interhemispheric reward signals converge onto a common integrator is the data from Malette and Miliaressis (1995); they obtained summation from bilateral MFB reward sites ranging from 40 to 90%.

Lesion studies also support the contention that the MFB shares interhemispheric anatomical connections with relation to reward. A general indication that the contralateral hemisphere is involved in MFB reward is the fact that animals which undergo extensive ablations of the whole forebrain in one hemisphere can still self-stimulate vigorously (Stellar et al., 1982).

Waraczynski's (2003) data also support this idea. She examined the effects of temporarily inactivating the extended amygdala on reward thresholds from the MFB. Contralateral infusions of lidocaine altered the rewarding value of the stimulation more in caudal MFB sites than in rostral ones. These results were interpreted as a potential indication that interhemispheric connections linking the central amygdala and the MFB occur posterior to the hypothalamus, in agreement with both the possibilities illustrated in Panels B and C of Figure 22.

Conclusion and future directions

In summary, our findings contribute to the unveiling of the circuitry linking the amygdaloid complex and the MFB with relation to reward. We suggest that the cortical amygdaloid nuclei play a crucial role in modulating reward signals elicited from both the LH and the VTA. These structures may thus be good candidates for the cell bodies of origin of the descending path hypothesis (see section 1.4.2.1). Another possibility is that amygdaloid signals exert their influence on MFB reward via a collateralized net of fibers. Our results additionally point to the existence of crossed connections in the context of this link.

The findings presented in this thesis give rise to a number of interesting future directions. A first priority is, in our view, that of pinpointing with greater accuracy the amygdaloid sub-nuclei involved in the modulation of MFB reward. Second, it is imperative to resolve the question of interhemispheric connectivity discussed above, that is, which of the three routes proposed in Figure 22 best represents the organization of crossed fibers underlying MFB-amygdaloid communication. A methodological improvement in order to achieve these goals concerns the lesion technique used to damage amygdaloid areas of interest. One possibility is the

use of lidocaine to temporarily inactivate target nuclei instead of permanently destroying brain tissue. This method has been recently used by Waraczynski and her team (Acheson, Waraczynski, and Perkins, 2000; Waraczynski, 2003; Waraczynski and Perkins, 2000) as well as by Ahn and Phillips (2002) with promising results. This technique offers multiple advantages, the most obvious one being that the secondary effects intrinsic to electrolytic damage (see above) do not have to be contended with. Another benefit of lidocaine inactivation lies in its temporary nature; a more direct correlation between cause and effect can be established as the rewarding value of the stimulation changes and then returns to baseline following the physiological activity of the anaesthetic. Finally, the use of lidocaine increases the power of an investigation by rendering it possible to test many different conditions within a single subject (see Waraczynski, 2003).

The results of this thesis open the possibility of exploring the link between amygdaloid and MFB reward fibers via techniques other than metabolic or lesion ones. One could, for instance, attempt collision between the cortical nucleus of the amygdala and the LH or between the amygdala and the VTA. This powerful technique has been briefly described in section 1.4.1.2 of this thesis' introduction. Recall that two sites can be shown to be functionally connected by delivering simultaneous impulses via implanted electrodes and behaviourally witnessing the effects of action potentials cancelling out (also see Miguelez and Bielajew, in press). The main difficulty associated with this approach is the likely development of seizures from repeated stimulation of the amygdaloid complex. A careful choice of stimulating parameters would have to be made in order to prevent this problem.

Finally, the metabolic data of our last investigation give rise to yet another future venue of

exploration. Recall that correlational analyses between rod density within lesion sites and MFB threshold shifts were interpreted as a potential indication that processes of regeneration may have been occurring at the first lesion site. It would be of particular interest to study the occurrence of these processes using microinjections of markers designed to visualize axonal outgrowth and synaptogenesis. For instance, immunostaining for an intraneuronal growth substance known as growth-associated protein has been shown to be an effective way of distinguishing elongating axons and synapse formation (see Ulfig, Setzer and Bohl, 2003). This technique could be used to see whether or not lesioned amygdaloid tissue of self-stimulating animals can show regenerative characteristics.

These potential avenues of exploration are just a few options among many that could be implemented to study the processes by which MFB reward signals undergo modulation by the amygdaloid complex. Regardless of the chosen paradigm or theoretical framework, it is our contention that the nature of the links between the amygdaloid complex and the MFB await further characterization and should become an important area of investigation for students of motivation and reward.

REFERENCES

Acheson, A., Waraczynski, M.M., Perkins, M. (2000). Lesions and inactivation implicate dorsolateral hindbrain in MFB self-stimulation. Physiology and Behavior, 70: 1-13.

Adamec, R. (1998). Amygdala kindling and rodent anxiety. In R. Corcoran and S. Moshe (Eds.). Kindling 5, New York: Plenum Press, pp. 327-348.

Adamec, R. (2001). Partial kindling and behavioral pathologies. International Review of Neurobiology, 45: 409-434.

Adamec, R., Shallow, T. (2000). Rodent anxiety and kindling of the central amygdala and nucleus basalis. Physiology and Behavior, 70: 177-187.

Adamec, R., Young, B. (2000). Neuroplasticity in specific limbic system circuits may mediate specific kindling induced changes in animal affect - implications for understanding anxiety associated with epilepsy. Neuroscience and Biobehavioral Reviews, 24: 705-723.

Aggleton, J.P. (1985). A description of intra-amygdaloid connections in old world monkeys. Experimental Brain Research, 57: 390-399.

Aguayo, A.J., Bray, G.M., Carter, D.A., Villegas-Perez, M.P., Vidal-Sanz, M., Rasminsky, M. (1990 a). Regrowth and connectivity of injured central nervous system axons in adult rodents. Acta Neurobiologica Experimentalis, 50: 381-389.

Aguayo, A.J., Bray, G.M., Rasminsky, M., Zwimpfer, T., Carter, D., Vidal-Sanz, M. (1990 b). Synaptic connections made by axons regenerating in the CNS of adult mammals. Journal of Experimental Biology, 153: 199-224.

Ahn, S., Phillips, A.G. (2002). Modulation by central and basolateral amygdalar nuclei of dopaminergic correlates of feeding to satiety in the rat nucleus accumbens and medial prefrontal cortex. The Journal of Neuroscience, 22: 10958-10965.

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: 1-39.

Amaral, D.G., Price, J.L., Pitkanen, A., Carmichael, S.T. (1992). Anatomical organization of the primate amygdala. In: J.P. Aggleton (Ed.). The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction. New York: Wiley-Liss, pp. 539-560.

Amaral, D.G., Veazey, R.B., Cowan, W.M. (1983). Some observations on hypothalamo-amygdaloid connections in the monkey. Brain Research, 252: 13-27.

Arvanitogiannis, A., Flores, C., Pfaus, J.G., Shizgal, P. (1996). Increased ipsilateral expression of Fos following lateral hypothalamic self-stimulation. Brain Research, 720: 148-154.

Arvanitogiannis, A., Waraczynski, M.M., Shizgal, P. (1996). Effects of excitotoxic lesions of the basal forebrain on MFB self-stimulation. Physiology and Behavior, 59: 795-806.

Balaban, P.M., Chase, R. (1989). Self-stimulation in snails. Neuroscience Research Communications, 4: 139-146.

Baron-Cohen, S., Ring, H.A., Bullmore, E.T., Wheelwright, S., Ashwin, C., Williams, S.C.R. (2000). The amygdala theory of autism. Neuroscience and Biobehavioral Reviews, 24: 355-364.

Bauman, M., Kemper, T. (1994). The Neurobiology of Autism. Baltimore: John Hopkins, n-p.

Ben-Ari, Y., Zigmond, R.E., Shute, C.C.D., Lewis, P.R. (1977). Regional distribution of choline acetyltransferase and acetylcholinesterase within the amygdaloid complex and stria terminalis system. Brain Research, 120: 435-445.

Bernadis, L.L., Bellinger, L. (1979). Feed efficiency in growth retarded rats with ventromedial hypothalamic lesions produced shortly after weaning. Physiology and Behavior, 23: 971-974.

Bianchin, M., Souza, T.M.E., Medina, J.H., Izquierdo, I. (1999). The amygdala is involved in the modulation of long-term memory, but not in working of short-term memory. Neurobiology, Learning, and Memory, 71: 127-131.

Bielajew, C. (1991). Distribution of cytochrome oxidase in response to rewarding brain stimulation: effect of different pulse durations. Brain Research Bulletin, 26: 379-384.

Bielajew, C., Bushnik, T., Konkle, A.T.M. Schindler, D. (2000). The substrate for brain-stimulation reward in the lateral preoptic area II. Connections to the ventral tegmental area. Brain Research, 881: 112-120.

Bielajew, C., Fouriezos, G. (1986). Cytochrome oxidase mapping of reward pathways. Society for Neuroscience Abstracts, 12: 932.

Bielajew, C., Jurgens, S., Fouriezos, G. (1987). The effects of pulse duration on refractory periods of neurons mediating brain-stimulation reward. Behavioural Brain Research, 24: 233-241.

Bielajew, C., Konkle, A., Fouriezos, G., Boucher-Thrasher, A. Schindler, D. (2001). The substrate for brain-stimulation reward in the lateral preoptic area: III. Connections to the lateral hypothalamic area. Behavioral Neuroscience, 115: 900-909.

Bielajew, C., Miguelez, M., Shiao, R. (2002). Electrolytic lesions of the cortical and adjacent nuclei in the amygdala differentially influence thresholds for rewarding medial forebrain bundle stimulation. Behavioral Neuroscience, 116: 660-671.

Bielajew, C., Shizgal, P. (1980). Dissociation of the substrates for medial forebrain bundle self-stimulation and stimulation-escape using a two-electrode stimulation technique. Physiology and Behavior, 25: 707-711.

Bielajew, C., Shizgal, P. (1982). Behaviorally derived measures of conduction velocity in the substrate for rewarding medial forebrain bundle stimulation. Brain Research, 237: 107-119.

Bielajew, C., Shizgal, P. (1986). Evidence implicating descending fibers in self-stimulation of the medial forebrain bundle. Journal of Neuroscience, 6: 919-929.

Bielajew, C., Trzcinska, M. (1998). Functional connections between medial prefrontal cortex and caudate-putamen in brain-stimulation reward of rats. Behavioral Neuroscience, 112: 1177-1186.

Bishop, M.P., Elder, S.T., Heath, R.G. (1963). Intracranial self-stimulation in man. Science, 140: 394-396.

Boyd, E.S, Gardner, L.C. (1962). Positive and negative reinforcement from intracranial stimulation of a teleost. Science, 136: 648-649.

Boyd, E.S., Gardner, L.C. (1967). Effect of some brain lesions on intracranial self-stimulation in the rat. American Journal of Physiology, 213: 1044-1052.

Boye, S. Rompré, P-P. (1996). Mesencephalic substrate of reward: axonal connections. Journal of Neuroscience, 16: 3511-3520.

Boyle, P.C., Storlien, L.H., Keesey, R.E. (1978). Increased efficiency of food utilization following weight loss. Physiology and Behavior, 21: 261-264.

Brady, D.R., Mufson, E.J. (1990). Amygdaloid pathology in Alzheimer's disease: Qualitative and quantitative analysis. Dementia, 1: 5-17.

Brothers, L. (1990). The social brain: A project for integrating primate behaviour and neurophysiology in a new domain. Concepts in Neuroscience, 1: 27-51.

Brown, V.J., Latimer, M.P., Winn, P. (1996). Memory for the changing cost of a reward is mediated by the sulenticular extended amygdala. Brain Research Bulletin, 39: 163-170.

Bruce, L.L., Neary, T.J. (1995). The limbic system of tetrapods: A comparative analysis of cortical and amygdalar populations. Brain, Behavior and Evolution, 46: 24-234.

Buckenham, K., Yeomans, J.S. (1993). An uncrossed tectopontine pathway mediates ipsiversive circling. Behavioral Brain Research, 54: 11-22.

Cain, D.P. (1992). Kindling and the amygdala. In J.P. Aggleton (Ed.). The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction. New York: Wiley-Liss, pp. 539-560.

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

Carlson, N.R. (1994). Physiology of Behavior. Third Ed. Boston: Allyn and Bacon, 704 p.

Cassell, M.D., Freedman, L.J., Shi, C. (1999). The intrinsic organization of the central extended amygdala. Annals of the New York Academy of Sciences, 877: 217-241.

Charney, D.S., Grillon, C. Bremner, J.D. (1998). The neurobiological basis of anxiety and fear: circuits, mechanisms, and neurochemical interactions (Part I). Neuroscientist, 4: 35-44.

Collier, T.J., Redmond, D.E., Roth, R.H., Elsworth, J.D., Taylor, J.R., Sladek, J.R. (1997). Metabolic energy capacity of dopaminergic grafts and the implanted striatum in parkinsonian nonhuman primates as visualized with cytochrome oxidase histochemistry. Cell Transplant, 6: 135-140.

Collins, D.R., Paré, D. (1999). Reciprocal changes in the firing probability of lateral and central medial amygdala neurons. Journal of Neuroscience, 19: 836-844.

Crichton, M. (1972). The Terminal Man. New York: Ballantine Books, 268 p.

Crosby E.C., Humphrey, T. (1941). Studies of the vertebrate telencephalon: II. The nuclear pattern of the anterior olfactory nucleus tuberculum olfactorium and the amygdaloid complex in adult man. Journal of Comparative Neurology, 74: 309-352.

Crosby E.C., Humphrey, T. (1944). Studies of the vertebrate telencephalon: III. The amygdaloid complex in the shrew *(Blarina brevicauda)*. Journal of Comparative Neurology, 81: 285-305.

Dauth, G.W., Dafny, N., Gilman, S. (1976). Unit responses in hypothalamus and mesencephalic reticular formation to acoustic stimuli and electrical stimulation of ipsi- and contralateral amygdala. Physiology and Behavior, 17: 621-629.

Davidson, R.J., Abercrombie, H., Nitschke, J.B., Putnam, K. (1999). Regional brain function, emotions and disorders of emotion. Current Opinion in Neurobiology, 9: 228-234.

Davidson, R.J. Irwin, W. (1999). The functional neuroanatomy of emotion and affective style. Trends in Cognitive Science, 3: 11-21.

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. New York: Wiley-Liss, pp. 255-306.

Deboer, T., Ross, R.J., Morrison, A.R., Sanford, L.D. (1999). Electrical stimulation of the amygdala increases the amplitude of elicited ponto-geniculo-occipital waves. Physiology and Behavior, 66: 119-124.

De Olmos, J.S. (1972). The amygdaloid projection field in the rat as studied with the cupric-silver method. In B.E. Eleftheriou (Ed.). The Neurobiology of the Amygdala, New York: Plenum Press, pp. 145-204.

De Olmos, J.S., Alheid, G.F., Beltramino, C.A. (1985). Amygdala. In G. Paxinos,(Ed.). The Rat Nervous System, Vol. 1, pp. 223-334.

De Olmos, J.S., Heimer, L. (1999). The concepts of the ventral striatopallidal system and extended amygdala. Annals of the New York Academy of Sciences, 877: 1-32.

DiRocco, R.J., Kageyama, G.H., Wong-Riley, M.T.T. (1989). The relationship between CNS metabolism and cytoarchitecture: a review of 14C-deoxyglucose studies with correlation to cytochrome oxidase histochemistry. Computer Medical Imaging Graphics, 13: 81-92.

Dong, H-W., Petrovich, G.D., Swanson, L.W. (2001). Topography of projections from amygdala to bed nuclei of the stria terminalis. Brain Research Reviews, 38: 192-246.

Dreifuss, J.J. (1972). Effects of electrical stimulation of the amygdaloid complex on the ventromedial hypothalamus. In B.E. Eleftheriou (Ed.). The Neurobiology of the Amygdala. New York: Plenum Press

Dreifuss, J.J., Murphy, J.T., Gloor, P. (1968). Contrasting effects of two identified amygdaloid efferent pathways on single hypothalamic neurons. Journal of Neurophysiology, 31: 237-348.

Durivage, A. Miliaressis, E. (1987). Anatomical dissociation of the substrates of medial forebrain self-stimulation and exploration. Behavioral Neuroscience, 101: 57-61.

Edmonds, D.E., Gallistel, C.R. (1974). Parametric analysis of brain stimulation reward in the rat: III. Effects of performance variables on the reward summation function. Journal of Comparative Physiological Psychology, 87: 876-883.

Egger, M.D. (1967). Response of hypothalamic neurons to electrical stimulation in the amygdala and the hypothalamus. Electroencephalography and Clinical Neurophysiology, 23: 6-15.

Egger MD. (1972). Amygdaloid-hypothalamic neurophysiological interrelationships. In B.E. Eleftheriou (Ed.). The Neurobiology of the Amygdala. New York: Plenum Press.

Eiden, L.E., Hökfelt, T., Brownstein, M.J., Palkovits, M. (1985). Vasoactive intestinal polypeptide afferents to the bed nucleus of the stria terminalis in the rat: An immunohistochemical and biochemical study. Neuroscience, 15: 999-1013.

Ellendorff, F., Parvizi, N. (1981). Neuroendocrinology of amygdaloid control of gonadotropine secretions. In Ben-Ari (Ed.). The amygdaloid complex, Amsterdam: Elsevier, pp. 239-250.

Erecinska, M., Silver, I.A. (1989). ATP and brain function. Journal of Cerebral Blood Flow and Metabolism, 9: 2-19.

Esposito, R.U., Porrino, L.J., Seeger, T.F., Crane, A.M., Everist, H.D., Pert, A. (1984). Changes in local cerebral glucose utilization during rewarding brain stimulation. Proceedings from the National Academy of Sciences USA, 81: 635-639.

Fellous, J. (1999). Neuromodulatory basis of emotion. Neuroscientist, 5: 283-294.

Fonberg, E. (1963). The inhibitory role of amygdala stimulation. Acta Biologoca Experimentalis, 23: 171-180.

Fonberg, E. (1967). The role of the amygdaloid nucleus in animal behavior. Progress in Brain Research, 22: 273-281.

Fonberg, E. (1969). The role of the hypothalamus and amygdala in food intake, alimentary motivation and emotional reactions. Acta Biologica Experimentalis, 29: 335-358.

Fonberg, E. (1972). Control of emotional behaviour through the hypothalamus and amygdaloid complex. Ciba Foundation Symposium, 8: 131-150.

Fonberg, E. (1973). The normalizing effect of lateral amygdalar lesions upon the dorsomedial amygdalar syndrome in dogs. Acta Neurobiologica Experimentalis, 33: 449-466.

Fonberg, E. (1974). Amygdala function within the alimentary system. Acta Neurobiologica Experimentalis, 34: 435-66.

Fonberg, E. (1975). The amygdala and ingestive behavior. In G.J. Mogenson, and A. Calaresu (Eds.). Neural Integration of Physiological Mechanisms and Behavior. Toronto: University of Toronto Press, Toronto, np.

Fonberg, E., Delgado, J.M.R. Avoidance and alimentary reactions during amygdala stimulation.(1961). Journal of Neurophysiology, 24: 651-664.

Fonberg, E., Sychowa, B. (1968). Effects of partial lesions of the amygdala in dogs. Acta Biologica Experimentalis, 28: 35-46.

Fouriezos, G., Bielajew, C., Pagotto, W. (1990). Task difficulty increases thresholds of rewarding brain stimulation. Behavioral Brain Research, 37: 1-7.

Friedman, B., Price, J.L. (1984). Fiber systems in the olfactory bulb and cortex: a study in adult and developing rats using the Timm method with the light and electron microscope. Journal of Comparative Neurology, 223: 88-109.

Gaffan, D. (1992). Amygdala and the memory of reward. In: J.P. Aggleton (Ed.). The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction. New York: Wiley-Liss, pp. 471-483.

Gallagher, M., Holland, P.C. (1992). Understanding the function of the central nucleus: Is simple conditioning enough? In J.P. Aggleton (Ed.). The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction. New York: Wiley-Liss, pp. 307-322.

Gallistel, C.R. (1974). Note on temporal summation in the reward system. Journal of Comparative Physiological Psychology, 87: 870-875.

Gallistel, C.R. (1978). Self-stimulation in the rat: quantitative characteristics of the reward pathways. Journal of Comparative and Physiological Psychology, 92: 977-998.

Gallistel, C.R. (1983). Self-stimulation. In J.A. Deutsch (Ed.). The Physiological basis of memory. New York: Academic Press, pp. 269-349.

Gallistel, C.R., Gomita, Y., Yadin, E., Campbell, K.A. (1985). Forebrain origins and terminations of the median forebrain bundle metabolically activated by rewarding stimulation or by reward-blocking doses of pimozide. Journal of Neuroscience, 5: 1246-1261.

Gallistel, C.R., Karreman, G., Reivic, M. (1977). 2-deoxyglucose uptake markks systems activated by rewarding brain stimulation. Brain Research Bulletin, 2: 149-152.

Gallistel, C.R., Leon, M., Lim, B.T., Sim, J.C., Waraczynski, M. (1996). Destruction of the medial forebrain bundle caudal to the site of stimulation reduces rewarding efficacy but destruction rostrally does not. Behavioral Neuroscience, 110: 766-790.

Gallistel, C.R., Shizgal, P., Yeomans, J.S. (1981). A portrait for the substrate for self-stimulation. Physiological Review, 88: 228-273.

Gallo, M., Roldan, G., Bures, J. (1992). Differential involvement of gustatory insular cortex and amygdala in the acquisition and retrieval of conditioned taste aversion in rats. Behavioral Brain Research, 52: 91-97.

German, D.C., Dalsass, M., Kiser, R.S. (1980). Electrophysiological examination of the ventral tegmental (A10) area in the rat. Brain Research, 181: 191-197.

Gloor, P. (1955). Electrophysiological studies on the connections of the amygdaloid nucleus in the cat. I. The neuronal organization of the amygdaloid projection system. Electroencephalography and Clinical Neurophysiology, 7: 223-242.

Gloor, P. Physiology of the limbic system. (1975). In J.K. Penry, and D.D. Daly (Eds.). Advances in Neurology, Vol. 11. New York: Raven Press.

Gloor, P., Murphy, J.T., Dreifuss, J.J. (1969). Electrophysiological studies of amygdalo-hypothalamic connections. Annals of the New York Academy of Sciences, 157: 629-641.

Gloor, P. (1992). Role of the amygdala in temporal lobe epilepsy. In J.P. Aggleton (Ed.). The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction. New York: Wiley-Liss, pp. 505-538.

Goodman, I.J., Brown, J.L. (1966). Stimulation of positively and negatively reinforcing sites in the avian brain. Life Sciences, 5: 693-704.

Gratton, A. Wise, R.A. (1988). Comparisons of refractory periods for medial forebrain fibers subserving stimulation-induced feeding and brain stimulation reward: a psychophysiological study. Brain Research, 438: 256-263.

Gray, T.S. (1999). Functional and antomical relationships among the amygdala, basal forebrain, ventral striatum, and cortex: an integrative discussion. Annals of the New York Academy of Sciences, 877: 439-445.

Grossman, S.P. (1964). Behavioral effects of chemical stimulation of the ventral amygdala. Journal of Comparative Physiological Psychology, 57: 29-36.

Guyenet, P.G., Agajanian, G.K. Antidromic identification of dopaminergic and other output neurons of the rat substantia nigra. Brain Research, 150: 69-84.

Halgren, E. (1992). Emotional neurophysiology of the amygdala within the context of human cognition. In E. Aggleton (Ed.). The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction. New York: Wiley-Liss, pp.191-228.

Hall, E. (1972). Some aspects of the structural organization of the amygdala. In B.E. Eleftheriou (Ed.). The Amygdaloid Complex. New York: Plenum Press, pp. 95-122.

Han, Z.S., Ju, G. (1990). Effects of electrical stimulation of the central nucleus of the amygdala and the lateral hypothalamic area on the oval nucleus of the bed nuclei of the stria terminalis and its adjacent areas in the rat. Brain Research, 536: 56-62.

Harley, C.A., Bielajew, C. (1992). A comparison of glycogen phosphorylase a and cytochrome oxidase histochemical staining in rat brain. The Journal of Comparative Neurology, 322: 377-389.

Hatfield, T., McGaugh, J.L. (1999). Norepinephrine infused into the basolateral amygdala posttraining enhances retention in a spatial water maze task. Neurobiology of Learning and Memory, 71: 232-239.

Helfer, V., Deransart, C., Marescaux, C., Depaulis, A. (1996). Amygdala kindling in the rat: Anxiogenic-like consequences. Neuroscience, 73: 971-978.

Hendry, S.H.C., Jones, E.G. (1986). Reduction in number of immunostained GABAergic neurons in deprived-eye dominance columns of monkey area 17. Nature, 320: 750-753.

Hevner, R.F., Liu, S., Wong-Riley, M.T.T. (1995). A metabolic map of cytochrome oxidase in the rat brain. Histochemical, densitometric, and biochemical studies. Neuroscience, 65: 313-342.

Holstege, G., Meiners, L., Tan, K. (1985). Projections of the bed nucleus of the stria terminalis to the mesencephalon, pons, and medulla oblongata in the cat. Experimental Brain Research, 58: 370-391.

Hopkins, D.A., Holstege, G. (1978). Amygdaloid projections to the mesencephalon, pons and medulla oblongata in the cat. Experimental Brain Research, 32: 529-547.

Horton, J.C., Hubel, D.H. (1981). Regular patchy distribution of cytochrome oxidase staining in primary visual cortex of macaque monkey. Nature, 292: 762-764.

Howell, D.C. (1992). Statistical Methods for Psychology (3d Ed.). New York: Wadsworth Publishing Company.

Howell, D.C. (1997). Statistical Methods for Psychology (4th Ed.). New York: Wadsworth Publishing Company.

Howell, D.C. (2001). Statistical Methods for Psychology (5th Ed.). New York: Wadsworth Publishing Company.

Hunt, G.E., McGregor, I.S. (1998). Rewarding brain stimulation induces only sparse fos-like immunoreactivity in dopaminergic neurons. Neuroscience, 83: 501-515.

Internet UTHSCSA Image Tool Analysis Package v. 2.0 (2001) San Antonio, TX.

Isaacson, R.L. (1982). The Limbic System. Second Ed. New York: Plenum Press, 327 p.

Jackson, W.J., Gardner, E.L. (1974). Modulation of hypothalamic ICSS by concurrent limbic stimulation. Physiology and Behavior, 12, 177-82.

Jameson, F. Pleasure: A political issue. (1983). In T. Bennett (Ed.). Formations of Pleasure, London: Rouledge and Kegan Paul, pp. 1-13.

Jankovic, B.D., Spector, N.H. (1986). In Plotnikoff, N.P., R.E. Faith, A.J. Murgo, R.A. Good (Eds.). Enkephalins and endorphins: Stress and the immune system. Plenum Press: New York.

Johnston, J.B. (1923). Further contributions to the study of the evolution of the forebrain. Journal of Comparative Neurology, 36: 143-192.

Jones, B., Mishkin, M. (1972). Limbic lesions and the problem of stimulus-reinforcement associations. Experimental Neurology, 36: 362-377.

Justesen, D.R., Sharp, J.C., Porter, R.B. (1963). Self-stimulation of the caudate nucleus by instrumentally naive cats. Journal of Comparative and Physiological Psychology, 56: 371-374.

Kandel, E.R., Schwartz, J.H., Jessell, T.M. (1991). Principles of Neural Science. (Third Ed.). Norwalk: Appleton and Lange, 1135 p.

Kane, F., Coulombe, D., Miliaressis, E. (1991a). Amygdaloid self-stimulation: A movable electrode mapping study. Behavioral Neuroscience, 105: 926-932.

Kane, F., Coulombe, D., Miliaressis, E. (1991b). Interactions between amygdaloid and hypothalamic self-stimulation: A re-examination. Behavioral Brain Research, 44: 169-183.

Kapp, B.S., Whalen, P.J., Supple, W.F., Pascoe, J.P. (1992). Amygdaloid contributions to conditioned arousal and sensory information processing. In J.P. Aggleton (Ed.). The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction. New York: Wiley-Liss, pp. 229-254.

Karnovsky, M.J., Roots, L. (1964). A direct coloring thiocholine method for cholinesterases. Journal of Histochemical Cytochemistry, 12: 219-221.

Kawata, M., Nakao, K., Morri, N., Kiso, Y., Yamashita, H., Imura, H., Sano, Y. (1985). Atrial natriuretic polypeptide: topographical distribution in the rat brain by radioimmunoassay and immunohistochemistry. Neuroscience, 16: 521-546.

Kennedy, C., DesRosiers, M., Jehle, J., Reivich, M., Sharpe, F., Sokoloff, L. (1975). Mapping of functional neural pathways by autoradiographic survey of local metabolic rate with [14C] deoxyglucose. Science, 187: 850-852.

King, B.M., Arceneaux, E.R., Cook, J.T., Benjamin, A.L., Alheid, G.F. (1996 a). Temporal lobe lesion-induced obesity in rats: An anatomical investigation of the posterior amygdala and hippocampal formation. Physiology and Behavior, 59: 843-848.

King, B.M., Cook, .T., Dallman, M.F. (1996 b). Hyperinsulinemia in rats with obesity-inducing amygdaloid lesions. American Journal of Physiology, 271: R1156-R1159.

King, B.M., Sam, H., Arceneaux, E.R., Kass, J.M. (1994). Effect on food intake and body weight of lesions in and adjacent to the posterolateral amygdala in rats. Physiology and Behavior, 55: 963-966.

Kiyatkin, E.A. (1989). Neurophysiology and neurochemistry of drug dependance: A review. International Journal of Neuroscience, 44: 283-316.

Kling, A.S., Brothers, L.A. (1992). The Amygdala and social behavior. In J.P. Aggleton (Ed.). The Amygdala: Neurobiological aspects of emotion, Memory, and mental dysfunction. New York: Wiley-Liss, pp. 353-377.

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

Koelle, G.B., Friedenwald, J.S. (1949). A histochemical method for localizing Cholinesterase activity. Proceedings from the Society of Experimental Biology, 70: 617-622.

Koikegami, H. (1963). Amygdala and other related limbic structures: experimental studies on the natomy and function: I. Anatomical researches with some neurophysiological observations. Acta Medica Biologica, 10: 161-277.

Konkle, A.T.M., Bielajew, C., Fouriezos, G., Trasher, A. (2001). Measuring threshold shifts for brain stimulation reward using the method of limits. Canadian Journal of Experimental Psychology, 55: 253-260.

Konkle, A.T.M., Wilson, P., Bielajew, C. (1999). Histochemical mapping of the substrate for brain-stimulation reward with glycogen phosphorylase. Journal of Neuroscience Methods, 93: 111-119.

Koob, G.F. (1998). Circuits, drugs, and drug addiction I. Neurobiological substrates for drug reinforcements-psychomotor stimulants, opiates, and sedative-hypnotics. In D.S. Goldstein, G. Eisenhofer, R. McCarty (Eds.). Advances in Pharmacology, Vol. 42, Catecholamines: Bridging Basic Science with Clinical Medicine, San Diego: Academic Press: pp. 979-982.

Koob, G.F. (1999). The role of the striatopallidal and extended amygdala systems in drug addiction. Annals of the New York Academy of Sciences, 877: 445-460.

Koob, G.F. (2001). Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology, 24: 97-129.

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

Krettek, J.E., Price, J.L. (1977a). The cortical projections of the mediocortical nucleus and adjacent thalamic nuclei in the rat. Journal of Comparative Neurology, 171: 157-192.

Krettek, J.E., Price, J.L. (1977b). Projections from the amygdaloid complex and adjacent olfactory structures to the entorhinal cortex and to the subiculum in the rat and cat. Journal of Comparative Neurology, 172: 723-752.

Krettek, J.E., Price, J. L. (1977c). Projections from the amygdaloid complex to the cerebral cortex and thalamus in the cat and rat. Journal of Comparative Neurology, 172: 687-722.

Krettek, J.E., Price, J.L. (1978a). Amygdaloid projections to subcortical structures within the basal forebrain and brainstem in the rat and cat. Journal of Comparative Neurology, 178: 225-254.

Krettek, J.E., Price, J.L. (1978b). A description of the amygdaloid complex in the rat and cat with observations on intra-amygdaloid axonal connections. Journal of Comparative Neurology, 178: 255-280.

LeDoux, J.E. (1992). Emotion and the amygdala. In: J.P. Aggleton (Ed.). The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction. New York: Wiley-Liss, pp. 339-351.

LeDoux, J.E. (2000). Emotion circuits in the brain. Annual Review of Neuroscience, 23: 155-184.

Lopez, L., Lorente, L., Arias, J., Gonzalezpardo, H., Cimadevilla, J., Arias, J.L. (1997). Changes of cytochrome oxidase activity in rat suprachiasmatic nucleus. Brain Research, 769: 367-371.

Lorens, S.A. (1976). Anatomical substrate of intracranial self-stimulation: contribution of lesion studies. In A. Wauquier and E.T. Rolls (Eds.). Brain-stimulation reward. New York: Elsevier, pp.41-50.

Loughlin, S.E., Fallon, J.H. (1983). Dopaminergic and non-dopaminergic projections to amygdala from substantia nigra and ventral tegmental area. Brain Research, 262: 334-338.

Lukaszewska, I., Korczynski, R., Kostarczyk, E., Fonberg, E. (1984). Food-motivated behavior in rats with cortico-basomedial amygdala damage. Behavioral Neuroscience, 98: 441-451.

Luskin, M.B., Price, J.L. (1983). The topographic organization of associational fibers of the olfactory system in the rat including centrifugal fibers to the olfactory bulb. Journal of Comparative Neurology, 216: 264-291.

Malette, J., Miliaressis, E. (1990). The notion of response invariance in trade-off studies of self-stimulation. Behavioral Brain Research, 40: 37-44.

Malette, J. Miliaressis, E. (1995). Interhemispheric links in brain stimulation reward. Behavioral Brain Research, 68: 117-137.

Mann, D.M.A (1992). The neuropathology of the amygdala in ageing and in dementia. In J.P. Aggleton (Ed.). The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction. New York: Wiley-Liss, pp. 575-593.

McDonald, A.J. (1982). Cytoarchitecture of the central amygdaloid nucleus in the rat. Journal of Comparative Neurology, 208: 401-418.

McDonald, A.J. (1992). Cell types and intrinsic connections of the amygala. In J.P. Aggleton (Ed.). The amygdala: Neurobiological aspects of emotion, memory, and mental dysfunction. New York: Wiley-Liss, pp. 67-96.

McDonald, A.J. (1998). Cortical pathways to the mammalian amygdala. Progress in Neurobiology, 55: 257-332.

McGaugh, J.L., Cahill, L., Roozendaal, B. (1996). Involvement of the amygdala in memory storage: Interaction with other brain systems. Proceedings from the National Academy of Sciences USA, 93: 13508-13514.

McGaugh, J.L., Introini-Collision, I.B., Cahill, L., Kim, M., and Liang, K.C. (1992). Involvement of the amygdala in neuromodulatory influences on memory storage. In: J.P. Aggleton (Ed.). The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction. New York: Wiley-Liss, pp. 431-452.

Merali, Z., McIntosh, J., Kent, P., Michaud, D., Anisman, H. (1998). Aversive and appetitive events evoke the release of corticotropin-releasing hormone and bombesin-like peptides at the central nucleus of the amygdala. The Journal of Neuroscience, 18: 4758-4766.

Miguelez, M., Bielajew, C. (2003). Mapping the neural substrate underlying brain stimulation reward with the behavioural adaptation of double-pulse methods: Review. Reviews in the Neurosciences, In Press.

Miguelez, M., Bielajew, C., Diotte, M. Shiao, R. (2001). Dynamic changes in cytochrome oxidase activity in the amygdala following lesions of rewarding sites in the lateral hypothalamus. Behavioral Brain Research, 119: 103-110.

Miguelez, M., Kentner, A., Deslauriers, K., Parkinson, M., Fouriezos, G., Bielajew, C. (2003). Interhemispheric involvement of the anterior cortical nuclei of the amygdala in rewarding brain stimulation. Brain Research, Accepted pending revisions.

Montgomery, R.B., Singer, G. (1975). Functional relationship of lateral hypothalamus and amygdala in control of eating. Pharmacology, Biochemistry and Behavior, 3: 905-907.

Morgane, P.J. (1962). Dissasociation of hypothalamic self-stimulation and primary feeding systems. Clinical Research, 10: 185.

Morgane, P.J., Kosman, A.J. (1960). Relationship of the middle hypothalamus to amygdala hyperphagia. American Journal of Physiology, 198: 1315-1318.

Mundl, W.J. (1980). A constant-current stimulator. Physiology and Behavior, 24: 991-993.

Murray, B., Shizgal, P. (1996a). Attenuation of medial forebrain bundle reward by anterior lateral hypothalamic lesions. Behavioral Brain Research, 75: 33-47.

Murray, B., Shizgal, P. (1996b). Physiological measures of conduction velocity and refractory period for putative reward-relevant MFB fibers arising in the rostral MFB. Physiology and Behavior, 59: 427-437.

Nakahara, D., Ishida, Y., Nakamura, M., Kuwahara, I., Todaka, K., Nishimori, T. (1999). Regional differences in desensitization of c-fos expression following repeated self-stimulation of the medial forebrain bundle in the rat. Neuroscience, 90: 1013-1020.

Nicholls, P., Butko, P. (1993). Protons, pumps, and potentials: Control of cytochrome oxidase. Journal of Bioenergetics and Biomembranes, 25: 137-143.

Nieuwenhuys, R, Geeraedts, L., Veening, J. (1982). The medial forebrain bundle of the rat. I. General Introduction. Journal of Comparative Neurology, 206: 49-81.

Nishijo, H., Onon, T., Uwano, T., Kondoh, T., Torii, K. (2000). Hypothalamic and amygdalar neuronal responses to various tastant solutions during ingestive behavior in rats. Journal of Nutrition, 130: 954S-959S.

Nitecka, L., Amerski, L., Narkiewicz, O. (1981). The organization of intraamygdaloid connections; an HRP study. Journal Hirnforsch, 22: 3-7.

Nobrega, J.N., Raymond, R., Di Stefano, L., Burnham, W. M.. (1993). Long-term changes in regional brain cytochrome oxidase activity induced by electroconvulsive treatment in rats. Brain Research, 605: 1-8.

Oades, R.D., Halliday, G.M. (1987). Ventral tegmental (A10) system: neurobiology. 1. Anatomy and connectivity. Brain Research Reviews, 12: 117-165.

Olds, M.E, Fobes, J.L. (1981). The central basis of motivation: Intracranial self-stimulation studies. Annual Review of Psychology, 32: 523-574.

Olds, J., Milner, P. (1954). Positive reinforcement produced by electrical stimulation of the septal area and other regions of the brain. Journal of Comparative and Physiological Psychology, 47: 419-428.

Olds, M.E., Olds, J. (1969). Effects of lesions in medial forebrain bundle on self-stimulation behavior. American Journal of Physiology, 217: 1253-1264.

Oomura, Y., Ono, T., Ooyama, H. (1970). Inhibitory action of the amygdala on the lateral hypothalamic area in rats. Nature, 228: 1108-1110.

Ottersen, O.P. (1982). Connections of the amygdala of the rat: IV. Corticoamygdaloid and intraamygdaloid connections as studied with axonal transport of horseradish peroxidase. Journal of Comparative Neurology, 205: 30-48.

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

Panagis, G., Miliaressis, E., Anagnostakis, Y., Spyraki, C. (1995). Ventral pallidum self-stimulation: a moveable electrode mapping study. Behavioral Brain Research, 68: 165-172.

Paré, D., Smith, Y. (1993). Distribution of GABA immunoreactivity in the amygdaloid complex of the cat. Neuroscience, 57: 1061-1076.

Paxinos, G., Watson, C. (1986). The rat brain in stereotaxic coordinates. New York: Academic Press.

Paxinos, G., Watson, C. (1998). The rat brain in stereotaxic coordinates. New York: Academic Press.

Pecson, R., Roth, D., Mark, V. (1969). Experimental temperature control of radiofrequency lesion size. Journal of Neurosurgery, 30: 703-707.

Petrovich, G.D., Canteras, N.S., Swanson, L.W. (2001). Combinatorial amygdalar inputs to hippocampal domains and hypothalamic behavior systems. Brain Research Reviews, 38: 247-289.

Phedon, In: Platon: Apologie de Socrate, Criton-Phedon, 1965, Paris: GF-Flammarion, p.107.

Pomonis, J.D., Levine, A.S., Billington, C.J. (1997). Interaction of the hypothalamic paraventricular nucleus and central nucleus of the amygdala in naloxone blockade of neuropeptide Y-induced feeding revealed by c-fos expression. Journal of Neuroscience, 17: 5175-5182.

Porrino, L.J., Esposito, R.U., Seeger, T.F., Crane, A.M., Pert, A., Sokoloff, L. (1984). Metabolic mapping of the brain during rewarding self-stimulation. Science, 224: 306-309.

Porrino, L.J., Huston-Lyons, D., Bain, G., Sokoloff, L., Kornetsky, C. (1990). The distribution of changes in local cerebral energy metabolism associated with brain stimulation reward of the medial forebrain bundle of the rat. Brain Research, 511: 1-6.

Powell, T.P.S., Cowan, W.M., Raisman, G. (1963). Olfactory relationships of the diencephalon. Nature, 199: 710-712.

Prewitt, C.M., Herman, J.P. (1998). Anatomical interactions between the central amygdaloid nucleus and the hypothalamic paraventricular nucleus of the rat: a dual tract-tracing analysis. Journal of Chemical Neuroanatomy, 15: 173-85.

Price, J.L. (1973). An autoradiographic study of complementary laminar patterns of termination of afferent fibers to the olfactory cortex. Journal of Comparative Neurology, 150: 87-108.

Price, J.L., Amaral, D.G. (1981). An autoradiographic study of the projections of the central nucleus of the monkey amygdala. Journal of Neuroscience, 1: 1242-1259.

Price, J.L., Russchen, F.T., Amaral, D.G. (1987). The limbic region. II: The amygdaloid complex. In A. Björklund, T. Hökfelt, L.W. Swanson (Eds.). Handbook of Chemical Neuroanatomy. Integrated systems of the CNS, Part I: Hypothalamus, Hippocampus, Amygdala, Retina, Vol. 5. Amsterdam: Elsevier, pp. 279-376.

Radwanska, K., Nikolaec, E., Knapska, E., Kaczmarek, L. (2002). Differential response of two subdivisions of lateral amygdala to aversive conditioning as revealed by c-Fos and P-ERK mapping. Neuroreport, 13: 2241-2246.

Rapin, I., Katzman, R. (1998). Neurobiology of autism. Annals of Neurology 43: 7-14.

Reilly, S. (1999). The parabrachial nucleus and conditioned taste aversion. Brain Research Bulletin, 48: 239-254.

Renaud, L.P. (1976). An electrophysiological study of amydalohypothalamic projections to the ventromedial nucleus of the rat. Brain Research, 105: 45-58.

Renaud, L.P., Hopkins, D.A. (1977). Amygdala afferents from the mediobasal hypothalamus: an electrophysiological and neuroanatomical study in the rat. Brain Research, 121: 201-13.

Reynolds, G.P. (1992). The amygdala and the neurochemistry of schizophrenia. In J.P. Aggleton (Ed.). The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction. New York: Wiley-Liss, pp. 561-574.

Robinson, S.R., Schousboe, A., Dringen, R. Magistretti, P., Coles, J., Hertz, L. Metabolic trafficking between neurons and glia. In P.R. Laming, E. Sykova, A. Reichenbach, G.I. Hatton, H. Bauer (Eds.). Glial cells: Their role in behaviour. Cambridge: Cambridge University Press, pp. 83-106.

Royer, S., Martina, M., Paré, D. (2000). Polarized synaptic interactions between intercalated neurons of the amygdala. Journal of Neurophysiology, 83: 3509-3518.

Royer, S., Paré, D. (2002). Bidirectional synaptic plasticity in intercalated amygdala neurons and the extinction of conditioned fear responses. Neuroscience, 115: 455-462.

Russchen, F.T. (1982a). Amygdalopetal projections in the cat: I. Cortical afferent connections. A study with retrograde and anterograde tracing techniques. Journal of Comparative Neurology, 206: 159-179.

Russchen, F.T. (1982b). Amygdalopetal projections in the cat: II. Subcortical afferent connections. A study with retrograde tracing techniques. Journal of Comparative Neurology, 207: 157-176.

Russchen, F.T., Price, J.L. (1984). Amygdalostriatal projections in the rat. Topographical organization and fiber morphology show using the lectin PHA-L as an anterograde tracer. Neuroscience Letters, 47: 15-22.

Saad, W.A., Paulino, M.A., Camargo, L.A.A., Renzi, A., Menani, J.V., Abrao-Saad, W. (1994). Effect of electrolytic and chemical lesion by ibotenic acid of the amygdala on salt intake. Brazilian Journal of Medical and Biological Research, 27: 2903-2907.

Salinas, J.A., Packard, M.G., McGaught, J.L. (1993). Amygdala modulates memory for changes in reward magnitude: reversible post-training inactivation with lidocaine attenuates the response to a reduction in reward. Behavioral Brain Research, 59: 153-159.

Sandner, G., Bielajew, C., Fouriezos, G. (1996). Bicuculline microinjections into the ventral tegmental area of the rat: alteration of self-stimulation thresholds and of cytochrome oxidase activity in the brain. Behavioural Brain Research, 79: 145-151.

Savander, V., Go, C.G., Ledoux, J.E., Pitkanen, A. (1996). Intrinsic connections of the rat amygdaloid complex: projections originating in the accessory basal nucleus. Journal of Comparative Neurology, 374: 291-313.

Scalia, F., Winans, F. (1975). The differential projections of the olfactory bulb in mammals. Journal of Comparative Neurology 161: 31-55.

Schenk, S., Shizgal, P. (1982). The substrates for lateral hypothalamic and medial prefrontal cortex self-stimulation have different refractory periods and show poor spatial summation. Journal of Physiology and Behavior, 28: 133-138.

Setlow, B., Gallagher, M., Holland, P.C. (2002). Disconnection of the basolateral amygdala complex and nucleus accumbens impairs appetitive Pavlovian second-order conditioned responses. Behavioral Neuroscience, 116: 267-275.

Shi, CJ., Davis, M. (1999). Pain pathways involved in fear conditioning measured with fear-potentiated startle: lesion studies. Journal of Neuroscience, 19: 420-430.

Shizgal, P. (1997). Neural basis of utility estimation. Current Opinion in Neurobiology, 7: 198-208.

Shizgal, P., Bielajew, C., Corbett, D., Skelton, R., Yeomans, J. (1980). Behavioral methods for inferring anatomical linkage between rewarding brain stimulation sites. Journal of Comparative Physiological Psychology, 94: 227-237.

Simmons, J.M., Ackerman, R.F., Gallistel, C.R. (1998). Medial forebrain bundle lesions fail to structurally and functionally disconnect the ventral tegmental area from many ipsilateral nuclei:

Implications for the neural substrate of brain stimulation reward. Journal of Neuroscience, 18: 8515-8533.

Singer, G., Montgomery, R.B. (1969). Functional relationship of lateral hypothalamus and amygdala in control of drinking. Physiology and Behavior, 4: 505-507.

Sokoloff, L., Kennedy, C., Smith, C.B. (1983). Metabolic mapping of functional activity in the central nervous system by measurement of local glucose utilization with Radioactive Deoxyglucose. In Handbook of Chemical Neuroanatomy, Vol. 1: Methods in Chemical Neuroanatomy. New York: Elsevier Science Publishers B.V.

Sokoloff, D., Reivich, M., Kennedy, C., DesRosiers, M. (1977). The 14-C-deoxyglucose method for measurement of local cerebral glucose utilization. Theory, procedure, and normal values in the conscious and anaesthetized albino rat. Journal of Neurochemistry, 28: 897-916.

SPSS. Statistical Analysis Package v. 11 (1998) Tulsa, OK: StatSoft.

Stark, P., Boyd, E.S. (1961). Electrical self-stimulation by dogs through chronically implanted electrodes in the hypothalamus. Federal Proceedings, 20: 328.

STATISTICA Statistical Analysis Package (1998) Tulsa, OK: StatSoft.

Stellar, J.R., Stellar, E. (1985). The Neurobiology of Motivation and Reward. New York: Springer-Verlag, 249 p.

Stuart, O.G., Porter, R.W., Adey, W.R. (1964). Hypothalamic unit activity II. Central and peripheral influences. Electroencephalography and Clinical Neurophysiology, 15: 248-258.

Stenger, J., Fournier, T., Bielajew, C. (1991). The effects of chronic ventromedial hypothalamic stimulation on weight gain in rats. Physiology and Behavior, 50: 1209-1213.

Swanson, L.W., Cowan, W.M. (1979). The connections of the septal area in the rat. Journal of Comparative Neurology, 172: 621-656.

Szabo, I., Rozowska, E., Kolta, P. (1972). Influence of contingent amygdaloid stimulation on lateral hypothalamic medial forebrain bundle self-stimulation. Physiology and Behavior, 6: 839-49.

Tomlinson, B.E., Corsellis, J.A.N. (1984). Ageing and the dementias. In J. Hume Adams, J.A.N. Corsellis, L.W. Duchen (Eds.). Greenfields Neuropathology, 4th Ed. London: Edward Arnold, pp. 951-1025.

Touzani, K., Velley, L. (1998). Electrical self-stimulation in the central amygdaloid nucleus after ibotenic acid lesion of the lateral hypothalamus. Behavioral Brain Research, 90: 115-24.

Trzcinska, M., Bielajew, C. (1998). Activation of reward-relevant neurons in the caudate-putamen influences the development of medial prefrontal cortex self-stimulation: a moveable electrode mapping study. Acta Neurobiologica Experimentalis, 58: 189-198.

Tsubokawa, T., Sutin, J. (1963). Mesencephalic influence upon the hypothalamis ventromedial nucleus. Electroencephalography and Clinical Neurophysiology, 15: 804-810.

Turner, B.H., Knapp, M.E. (1976). Projections of the nucleus and tracts of the stria terminalis following lesions at the level of the anterior commissure. Experimental Neurology, 51: 468-479.

Ulfig, N., Setzer, M., Bohl, J. (2003). Ontogeny of the human amygdala. In P. Shinnick-Gallagher, A. Pitkanen, A. Shekhar, L. Cahill (Eds.). The Amygdala in Brain Function, Basic and Clinical Approaches. Annals of the New York Academy of Sciences, 985, pp. 22-31.

Vachon, M.P., Miliaressis, E. (1992). Dorsal diencephalic self-stimulation: a moveable electrode mapping study. Behavioral Neuroscience, 106: 981-91.

Valenstein, E.S. (1964). Problems of measurement and interpretation with reinforcing brain stimulation. Psychological Review 71: 415-436.

Valenstein, E.S. (1973). Brain Control: A Critical Examination of Brain Stimulation and Psychosurgery. New York: Wiley and Sons.

Valenstein, E.S., Cox, V.C, Kakolewski, J.W. (1970). A reexamination of the role of the hypothalamus in motivation. Psychological Reveiw, 77: 16-31.

Van Atta, L., Sutin, J. (1971). The response of single lateral hypothalamic neurons to ventromedial nucleus and limbic stimulation. Physiology and Behavior, 6: 523-536.

Van der Kooy, D., Koda, L.Y., McGinty, J.F., Gerfen, C.R., Bloom, F.E. (1984). The organization of projections from the cortex, amygdala, and hypothalamus to the nucleus of the solitary tract in rat. Journal of Comparative Neurology, 224: 1-24.

Van der Zee, E.A., Luiten, P.G.M. (1999). Muscarinic acetylcholine receptors in the hippocampus, neocortex and amygdala: a review of immunocytochemical localization in relation to learning and memory. Progress in Neurobiology, 58: 409-471.

Veening, J.G, Swanson, L.W., Cowan, W.M, Nieuwenhuys, R., Geeraedts, L.M.G. (1982). The medial forebrain bundle of the rat. II. An autoradiographic study of the topography of the major descending and ascending components. The Journal of Comparative Neurology, 206: 82-108.

Wakefiled, C. (1980). The topographical organization and laminar origin of some cortico-amygdaloid connections. Neuroscience Letters, 20: 21-34.

Waraczynski, M. (2003). Lidocaine inactivation demonstrates a stronger role for central versus medial extended amygdala in medial forebrain bundle self-stimulation. Brain Research, 962: 180-198.

Waraczynski, M., Carlton, E., Perkins, M. (1998). Midbrain periaqueductal lesions do not degrade medial forebrain bundle stimulation reward. Behavioral Brain Research, 95: 167-177.

Waraczynski, M., Ng Cheong Ton, M., Shizgal, P. (1990). Failure of amygdaloid lesions to increase the threshold for self-stimulation of the lateral hypothalamus and ventral tegmental area. Behavioral Brain Research, 40: 159-168.

Waraczynski, M., Perkins, M. (1998). Lesions of pontomesencephalic cholinergic nuclei do not substantially disrupt the reward value of median forebrain bundle stimulation. Brain Research, 800: 154-169.

Waraczynski, M., Perkins, M. (2000). Temporary inactivation of the retrorubral fields decreases the rewarding effect of medial forebrain bundle stimulation. Brain Research, 885: 154-165.

Waraczynski, M., Perkins, M., Acheson, A. (1999). Lesions of midline midbrain structures leave medial forebrain bundle self-stimulation intact. Behavioral Brain Research, 103: 175-184.

Waraczynski, M., Shizgal, P. (1995). Self-stimulation of the MFB following parabrachial lesions. Journal of Physiology and Behavior, 58: 559-566.

Warembourg, M. (1981). Localization of steroid receptors in the amygdaloid complex. In Y. Ben-Ari (Ed.). The amygdaloid complex. Amsterdam: Elsevier, pp. 203-208.

Watanabe, T., Yamamoto, R., Maeda, A., Nakagawa, T., Minami, M., Satoh, M. (2002). Effects of excitotoxic lesions of the central or basolateral nucleus of the amygdala on naloxone-precipitated withdrawal-induced conditioned place aversion in morphine-dependent rats. Brain Research, 958: 423-428.

Wendt, R.H., Adey, W.R. (1960). A study of evoked unit activity in the hypothalamus. Anatomy Rec, 136:301.

Whitehurst, L. (2000). Recovery of rewarding effectiveness of lateral hypothalamic self-stimulation following radiofrequency lesions. PhD Thesis, University of Ottawa, Canada.

Wiegand, S.J., Price, J.L. (1980). Cells of origin of the afferent fibers to the median eminence in the rat. Journal of Comparative Neurology, 192: 51-77.

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

Winer, B.J., Brown, D.R., Michels, K.M. (1991). Statistical Principles in Experimental Design (3d Ed.). New York: McGraw Hill Inc.

Wong-Riley, M.T.T. (1979). Changes in the visual system of monocularly sutured or enucleated cats demonstratable with cytochrome oxidase histochemistry. Brain Research, 171: 11-28.

Wong-Riley, M.T.T. (1989). Cytochrome oxidase: an endogenous metabolic marker for neuronal activity. TINS, 12: 94-101.

Wong-Riley, M., Carroll, E.W. (1984). Effect of impulse blockage on cytochrome oxidase activity in monkey visual system. Nature, 307: 262-264.

Wurtz, R.H., Olds, J. (1963). Amygdaloid stimulation and operant reinforcement in the rat. Journal of Comparative Neurology, 56: 941-949.

Yadin, E., Guarini, V., Gallistel, C.R. (1983). Unilaterally activated systems in rats self-stimulating at sites in the medial forebrain bundle, medial prefrontal cortex, or locus coeruleus. Brain Research, 266: 39-50.

Yeomans, J.S. (1975). Quantitative measurements of neural post-stimulation excitability with behavioral methods. Physiology and Behavior, 15: 593-602.

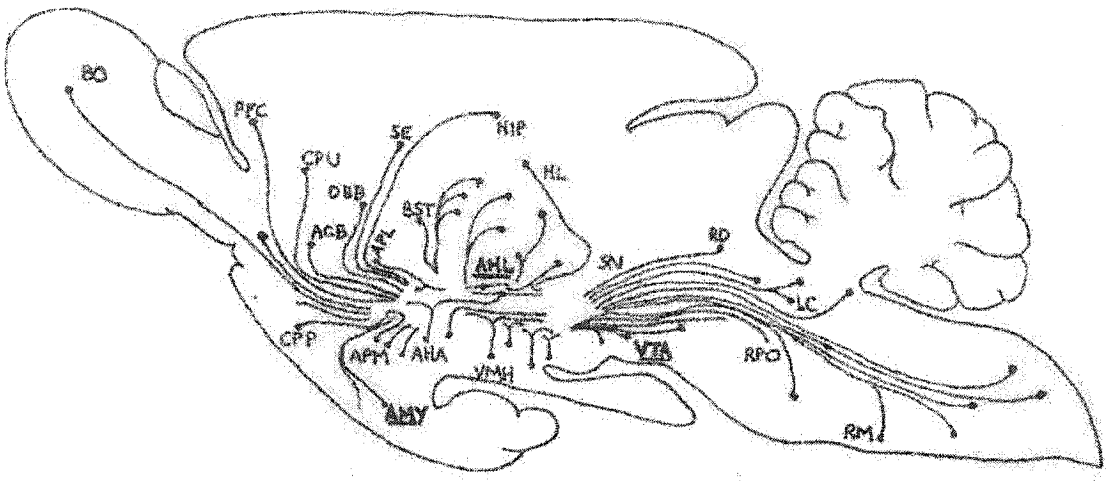
Yeomans, J.S. (1990). Principles of Brain Stimulation. New York: Oxford University Press, 182 p.

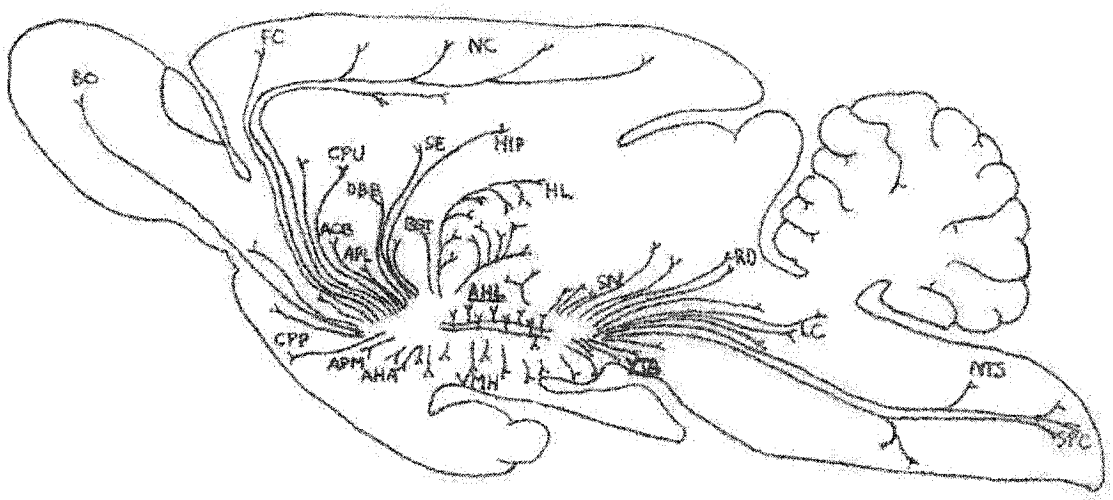
Yeomans, J.S, Maidment, N.T., Bunney, B.S. (1988). Excitability properties of medial forebrain bundle axons of A9 and A10 dopamine cells. Brain Research, 450: 86-93.

Appendix 1. Simplified representation of a sagittal section of the rat brain with MFB connections. The top panel (A) illustrates inputs to the MFB whereas the bottom panel (B) illustrates its outputs. This figure is adapted from Nieuwenhuys et al. (1982).

Abbreviations:

ACB: Nucleus accumbens
AHA: Anterior hypothalamus
AHL: Lateral hypothalamus
AMY: Amygdaloid complex
APL: Lateral preoptic area
APM: Medial preoptic area
BO: Olfactory bulb
BCA: Bed nucleus of the anterior commissure
CPA: Periamygdaloid cortex
CPP: Prepiriform cortex
CPU: Caudatoputamen
DBB: Nucleus of the diagonal band Broca
FC: Frontal cortex
HIP: Hippocampus
HL: Lateral habenula
LC: Locus coeruleus
NC: Neocortex
PFC: Prefrontal cortex
RD: Dorsal raphe
RM: Raphe magnus
RPO: Raphe pontis
SE: Septum
SN: Substantia nigra
SO: Superoptic nucleus
SPC: Spinal cord
VMH: Ventromedial hypothalamus
VTA: Ventral tegmental area
ZI: Zona incerta


BO
PFC
SE
HIP
CPU
DBB
HL
BST
ACB
APL
AHL
SN
RD
LC
CPP
APM
AHA
VMH
VTA
RPO
AMY
RM


FC
NC
BO
CPU
SE
HIP
DBB
HL
ACB
APL
BST
AHL
SN
RD
LC
CPP
APM
AHA
VMH
VTA
NTS
MPC

Appendix 2. Sample of studies examining the impact of electrolytic lesions on brain stimulation reward. Only investigations using threshold determinations as a scaling procedure for the rewarding effect of BSR are considered. Note that post-lesion effect are only considered as such when the post-lesion rewarding value of the stimulation has changed more than 0.10 $\log_{10}$ units from baseline. The arrows pointing up and down refer to the direction of the post-lesion effect: increase and decrease in the rewarding value of the stimulation respectively. Note also that sites located less than 4.5 mm caudal to bregma are called "LH", whereas sites at least 4.5 mm caudal to bregma are called "VTA".

Ref	Scaling & lesion proce-dure	Lesion site	Stimulation site	Direction and magnitude of lesion effects	Time course and duration of lesion effects
Acheson et al. (2000)	-Rate-frequency thresholds -Serial bilateral lesions at 14 days interval, 14 test days in each hemisphere following each lesion	Motor nucleus of the trigeminal nerve	LH ipsilateral to lesion	**4 rats** - no effect **Rat Mo2** -effect of the first lesion (made to contralateral side) is maintained **Rat Mo6** -1 reward (0.2 $\log_{10}$ units)	 -effect 1 day after lesion, maintained throughout the 14 post-lesion test days -effect 2 days after lesion, maintained throughout the 14 post-lesion test days
			----------	----------	----------
			LH contralateral to lesion	**Rat Mo2** -1 reward (0.2 $\log_{10}$ units) **Rat Mo6** -effect of the first lesion (made to the ipsilateral side) is maintained	-effect 7 days after lesion, maintained throughout the 14 post-lesion test days -effect 1 day after lesion, maintained throughout the 14 post-lesion test days
			----------	----------	----------
			VTA ipsilateral to lesion	**Rat Mo2** -1 reward (0.15 $\log_{10}$ units) **Rat Mo5** -1 reward (0.15 $\log_{10}$ units)	-effect 2 days after lesion, lasts 2 out of 14 post-lesion test days -effect 1 day after lesion, maintained throughout the 14 post-lesion test days
			----------	----------	----------
			VTA contralateral to lesion	**Rats Mo2 and Mo6** -no effect	

Ref	Scaling & lesion procedure	Lesion site	Stimulation site	Direction and magnitude of lesion effects	Time course and duration of lesion effects
Gallistel et al. (1996)	-Rate-frequency thresholds at 3 different currents -3 progressively larger lesions at 4 days interval, 3 test days after each lesion	Posterior LH	Posterior LH (stimulation site)	**4 rats** -↓ reward (± 0.10 log_{10} units, effect greater at lower currents)	-effect 1 day after lesion, maintained throughout the 3 post-lesion test days
	---	Posterior LH	Posterior LH (stimulation site)	**1 rat** -↓ reward (completely abolishes BSR for the first 8 days at all currents)	-effect 1 day after lesion, abolishes BSR for first 8 days, progressive recovery over subsequent 8 days, stabilizes at 80% of baseline throughout final 19 post-lesion test days
	---	VTA	Dorsomedial LH ipsilateral to lesion	**2 rats** -no effect **Rat ML8** -↓ reward (maximum effect: 0.30 log_{10} units at middle current)	-effect 1 day after first lesion, increased with subsequent lesions over the 14 test days following each lesion
	--- -3 to 5 progress. larger lesions at 7 to 14 days interval, testing for 7 to 14 days after each lesion	---	---	---	---

Ref	Scaling & lesion proce-dure	Lesion site	Stimulation site	Direction and magnitude of lesion effects	Time course and duration of lesion effects
Gallist el et al. (1996) (contin ued)		VTA	Anterior LH ipsilateral to lesion	**5 rats** -no effect	
				Rat RX8 -↓ reward (0.10 $\log_{10}$ units at lowest current)	-effect 1 day after first lesion, increased with subsequent lesions over the 14 test days following each lesion
				Rat RX17 -↓ reward (0.25 $\log_{10}$ units at middle current)	-effect 1 day after first lesion, increased with subsequent lesions over the 14 test days following each lesion
				Rat RX 11 -↓ reward (0.25 $\log_{10}$ units at middle current)	-effect 1 day after first lesion, increased with subsequent lesions over the 14 test days following each lesion
				Rat RX6 -↓ reward (0.25 $\log_{10}$ units at middle current)	-effect 1 day after first lesion, increased with subsequent lesions over the 14 test days following each lesion

Ref	Scaling & lesion procedure	Lesion site	Stimulation site	Direction and magnitude of lesion effects	Time course and duration of lesion effects
Murray & Shizgal (1996)	-Rate-frequency thresholds at 3 different currents -1 lesion, testing for 14 to 56 days post-lesion	Posterior LH	Anterior LH ipilateral to lesion	**6 rats** -no effect **Rat J1** -↓ reward (maximum effect: ± 0.40 log_{10} units at all tested currents) **Rat J2** -↓ reward (maximum effect: 0.40 log_{10} units at low and middle currents) **Rat F3** -↓ reward (0.20 log_{10} units at lowest current) **Rat F8** -↓ reward (0.20 log_{10} units at all tested currents) **Rat J3** -↓ reward (0.15 log_{10} units at all tested currents)	-effect 14 days after lesion (not tested before this point), declines and stabilizes within 0.10 log_{10} units of baseline at day 8 post-lesion, this effect is maintained throughout the subsequent 34 post-lesion test days -effect 1 days after lesion, declines and stabilizes within 0.10 log_{10} units of baseline at day 8 post-lesion, this effect is maintained throughout the subsequent 13 post-lesion test days -effect 5 days after lesion (not tested before this point), maintained throughout the subsequent 20 post-lesion test days -effect 1 day after lesion, declines and returns to baseline at day 10 post-lesion, maintained at baseline throughout the subsequent 17 post-lesion test days -effect 1 day after lesion, declines and returns to baseline at day 3 post-lesion, maintained at baseline throughout the subsequent 10 post-lesion test days

Ref	Scaling & lesion proce-dure	Lesion site	Stimulation site	Direction and magnitude of lesion effects	Time course and duration of lesion effects
Murray & Shizgal (1996) (contin ued)		VTA	Anterior LH ipsilateral to lesion	**11 rats** -no effect	
				Rat J2 -↓ reward (maximum effect: 0.30 $\log_{10}$ units for lowest current tested)	-effect 1 day after lesion, declines and returns to baseline at day 15 post-lesion, maintained at baseline throughout the subsequent 15 post-lesion test days
				Rat J13 -↓ reward (maximum effect: 0.40 $\log_{10}$ units for lowest current tested)	-effect 1 day after lesion, declines and stabilizes within 0.05 $\log_{10}$ units of baseline at day 20 post-lesion, maintained throughout the subsequent 10 post-lesion test days
				Rat F8 -↓ reward (maximum effect: 0.20 $\log_{10}$ units at all currents tested)	-effect 1 day after lesion, declines and returns to baseline at day 10 post-lesion, maintained throughout the subsequent 22 post-lesion test days
				Rat J10 -↑ reward (maximum effect: 0.20 $\log_{10}$ units at all currents tested)	-effect 1 day after lesion, return to baseline at day 20 post-lesion, maintained throughout the subsequent 10 post-lesion test days

Ref	Scaling & lesion procedure	Lesion site	Stimulation site	Direction and magnitude of lesion effects	Time course and duration of lesion effects
Simmons et al. (1998)	-Rate-frequency thresholds -1 lesion, testing for 21 days post-lesion	LH	VTA ipsilateral to lesion	**7 rats** -no effect **8 rats** -↓ reward (± 0.10 $\log_{10}$ units) **4 rats** ↓ reward (± 0.2 $\log_{10}$ units)	 -time course and duration not specified -time course and duration not specified
Waraczynski et al. (1990)	-Rate-frequency thresholds at 3 different currents -4 progressively larger lesions, at 4 days interval, 3 test days after each lesion	Amygdaloid complex nuclei: Lateral, Central, Basolateral, BNST	LH and VTA ipsilateral to lesion	**11 rats** -no effect	

Ref	Scaling & lesion procedure	Lesion site	Stimulation site	Direction and magnitude of lesion effects	Time course and duration of lesion effects
Waraczynski et al. (1995)	-Rate-frequency at 3 different currents -1 lesion, testing for 21 days post-lesion	Parabrachial nucleus	LH ipsilateral to lesion	**4 rats** -no effect **Rat 7** -↑ reward (0.10 $\log_{10}$ units at lowest current) **Rat 19** -↑ reward (0.10 $\log_{10}$ units at lowest current) **Rat 14** -↓ reward (0.10 $\log_{10}$ units at two highest currents tested) **Rat 18** -no effect **Rat 20** -↓ reward (maximum effect: 0.20 $\log_{10}$ units at all tested currents) **Rat 21** -↓ reward (maximum effect ranging from 0.10 to 0.25 $\log_{10}$ units at all tested currents)	-effect 1 day after lesion, declines and returns to baseline at day 5 post-lesion, maintained throughout the subsequent 15 post-lesion test days -effect 1 day after lesion, maintained throughout the subsequent 20 post-lesion test days -effect 1 day after lesion for highest current, returns to baseline at day 16 post-lesion, maintained throughout the subsequent 5 post-lesion test days -effect 1 day after lesion, increases progressively and is maintained throughout the 21 post-lesion test days -effect 5 days after lesion, increases progressively and is maintained throughout the 21 post-lesion test days

Ref	Scaling & lesion proce-dure	Lesion site	Stimulation site	Direction and magnitude of lesion effects	Time course and duration of lesion effects
Warac zynski et al. (1995) (contin ued)			VTA ipsilateral to lesion	**4 rats** -no effect **Rats 7 and 14** -no effect **Rat 18** -↓ reward (maximum effect: 0.30 $\log_{10}$ units at all tested currents) **Rat 20** -↓ reward -(0.10 to 0.20 $\log_{10}$ units at all tested currents)	-effect starts appearing 5 days after lesion, increases progressively and is maintained throughout the 21 post-lesion test days -effect starts appearing 1 day after lesion, increases progressively and is maintained throughout the 21 post-lesion test days

Ref	Scaling & lesion procedure	Lesion site	Stimulation site	Direction and magnitude of lesion effects	Time course and duration of lesion effects
Waraczynski et al. (1998)	-Rate-frequency at 3 different currents -1 lesion, testing for 21 to 28 days post-lesion	Dorsal raphe	LH ipsilateral to lesion	**1 rat** -no effect **Rat DR5** -no effect **Rat DR6** -↑ reward (maximum effect: 0.20 $\log_{10}$ units at lowest current)	-effect 1 day after lesion, maintained throughout the 21 post-lesion test days
			---	---	---
			VTA ipsilateral to lesion	**Rat DR5** -↑ reward (±0.10 $\log_{10}$ units at highest and lowest currents) **Rat DR6** -↑ reward (maximum effect: 0.20 $\log_{10}$ units at lowest current)	-effect 1 day after lesion, maintained throughout the 21 post-lesion test days -effect starts appearing 1 day after lesion, increases and is maintained throughout the 21 post-lesion test days
			---	---	---

Ref	Scaling & lesion procedure	Lesion site	Stimulation site	Direction and magnitude of lesion effects	Time course and duration of lesion effects
Waraczynski et al. (1998) (continued)		Central gray	LH ipsilateral to lesion	**7 rats** -no effect **CG12** -no effect **CG16** -no change	
			---	---	---
			VTA ipsilateral to lesion	**7 rats** -no effect **CG12** -↓ reward (maximum effect: 0.10 $\log_{10}$ units at all tested currents) **CG16** -↓ reward (maximum effect: 0.30 $\log_{10}$ units at middle current)	-effect 21 days after lesion, maintained for 7 subsequent post-lesion test days -effect starts appearing 7 days after lesion, increases and is maintained throughout the 25 post-lesion test days

Appendix 3. Summary table of the equivalencies existing between McDonald's (1998) nomenclature of the rat amygdaloid nuclear groups and the terminology employed by Paxinos and Watson (1996, 1998).

MCDONALD (1998)	PAXINOS & WATSON (1986, 1998)
Basolateral nuclear group	
Lateral nucleus	Lateral amygdaloid nucleus
	Dorsolateral part (LaDL)
	Ventrolateral part (LaVL)
	Ventromedial part (LaVM)
Basal nucleus	Basolateral amygdaloid nucleus
	Anterior part (BLA)
	Posterior part (BLP)
	Ventral part (BLV)
Accessory basal nucleus	Basomedial amygdaloid nucleus
	Anterior part (BMA)
	Posterior part (BMP)
Corticomedial nuclear group	
Anterior cortical nucleus	Anterior cortical amygdaloid nucleus (ACo)
Posterior cortical nucleus	Posteromedial cortical nucleus (PMCo)
Bed nucleus of the lateral olfactory tract	Bed nucleus of the accessory olfactory tract (BAOT)
Periamygdaloid complex	Posterolateral subdivision of the cortical nucleus (C2) (PLCo)

MCDONALD (1998)	PAXINOS & WATSON (1986, 1998)
Centromedial nuclear group	
Medial nucleus	Medial amygdaloid nucleus
	Anterodorsal part (MeAD)
	Anteroventral part (MeAV)
	Posteroventral part (MePV)
Central nucleus	Central amygdaloid nucleus
	Capsular part (CeC)
	Lateral division (CeL)
	Medial division (CeM)
Bed nucleus of the stria terminalis	Bed nucleus of the stria terminalis (BST)
	Fusiform part (Fu)
	Supracapsular part (BSTS)
	Intraamygdaloid division (BSTIA)
	Lateral division (BSTL)
	Dorsal part of the lateral division (BSTLD)
	Intermediate part of the lateral division (BSTLI)
	Lateral division of the juxtacapsular part (BSTLJ)
	Posterior part of the lateral division (BSTLP)
	Ventral part of the lateral division
	Medial division (BSTM)

MCDONALD (1998)	PAXINOS & WATSON (1986, 1998)
	Anterior part of the medial division (BSTMA)
	Posterior part of the medial division (BSTMP)
	Posterointermediate part of the medial division (BSTMPI)
	Posterolateral part of the medial division (BSTMPL)
	Posteromedial part of the medial division (BSTMPM)
	Ventral part of the medial division (BSTMV)
Other amygdaloid nuclei	
Amygdalohippocampal area	Amygdalohippocampal area
	Anterolateral part (AhiAL)
	Posteromedial part (AhiPM)
Intercalated nuclei	Intercalated nuclei of the amygdala (I)