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A Physiological and Behavioral Study of Emotional Stress in the Rat Medial Frontal Cortex

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VITA

The author, Robert J. Frysztak, was born on May 7th, 1961 in Chicago, Illinois to Jerome and Therese Frysztak. His secondary education was received at Glenbard North High School in Carol Stream, Illinois, from which he graduated in June of 1979. In August of that year, he entered Bradley University in Peoria, Illinois. He graduated Cum Laude with a Bachelor of Science degree in Biology in May of 1983.

In August of 1984, he entered the Department of Anatomy of the Graduate School of Loyola University of Chicago. While in the department, he was the recipient of the Loyola University Basic Science Fellowship from 1985 to 1987. In May of 1988 he received first place in the 18th Annual Sigma Xi Graduate Research Forum. In August of 1988, he was awarded the Loyola University Dissertation Fellowship. Along with his advisor, Edward J. Neafsey, Ph.D., he was awarded a Loyola University Pott's Estate Fund Grant in October of 1988 entitled *"The role of the rat medial frontal cortex in cardiovascular and respiratory responses to emotional stress."* He also taught in the medical school gross anatomy and neuroscience courses, and tutored medical gross anatomy. In 1988, he was elected into Sigma Xi, the scientific research society. Additionally, he served as the graduate student representative on the Department of Anatomy Graduate Admissions Committee in 1987 and 1988. Membership in professional societies

includes the Society for Neuroscience, Sigma Xi and the American Association for the Advancement of Science.

Robert has been awarded a fellowship from the Department of Medical Anatomy at Texas A&M University in College Station, Texas. He will study the functional brain circuitry related to learning and memory utilizing the 2-Deoxyglucose technique under the supervision of Dr. Francisco Gonzalez-Lima.

Robert is married to Beth Ann Young, and they are the proud parents of a beautiful 1 year-old girl, Kaitlyn Grace.

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To my mother, who has waited so patiently to read this.

CHAPTER I

INTRODUCTION

Until the 1930's, the frontal lobes were thought to house the higher intellectual capacities of man, including abstract conceptualization, foresight, ethical behavior and self awareness (see review by Warren & Kolb, 1978). In 1931, Jacobsen first described how large, bilateral frontal lesions in chimpanzees caused only a relatively subtle deficit in delayed response tasks and eliminated emotional outbursts (Jacobsen, 1935; Jacobsen et al., 1935). In 1939, Hebb reported that patients with frontal lobe removals (for treatment of epilepsy) did NOT score lower on standardized IQ tests. Together these studies led to a re-examination of frontal lobe function in both humans and non-humans.

Several reviews on the function of prefrontal cortex have focused on the behavioral (Konorski et al., 1972; Fuster, 1980; Rosenkilde, 1979), electrophysiological (Fuster, 1980) and clinical (Damasio, 1983; Fuster, 1980; Stuss & Benson, 1984, 1986) aspects of the frontal lobes. Although, a consensus on the function of the prefrontal cortex has not been reached, Fuster's (1980) view is representative of current thinking. He argues that the overall function of the prefrontal cortex is to "provide flexibility and unity in behavior by providing a 'temporal structure' in which behavior is organized into a meaningful whole." Lesions of the frontal cortex disrupt this temporal ordering, and the resulting deficits are both behavioral and psychological. The behavioral deficits in man include impaired response inhibition (Luria, 1969; Luria & Homskaya, 1964; Milner, 1964), impaired social behavior (Blumer & Benson, 1975), reduced behavioral spontaneity (Milner, 1964; Kolb & Milner, 1981; Kolb & Taylor, 1981),

impaired spatial orientation (Milner, 1965), and aphasia (Brown, 1972; Damasio, 1981). Psychological deficits include mood changes and character changes (Nauta, 1971). Nauta (1971) attributed these behavioral and psychological detriments following frontal cortical lesions to "inadequate internalization" of the individual's actions, resulting in "abnormal inflexibility of the program or excessive vulnerability to interfering events."

Non-human data on the frontal cortex has been derived primarily from lesion studies with primates and rats, investigating many of the same (or similar) behavioral deficits seen in man. These behavioral deficits include impairments in: response inhibition, temporal ordering, spatial orientation, social and affective states, behavioral spontaneity, complex ordered motor skills (such as nest building), and habituation (see review in Kolb, 1984). In virtually all cases, the deficits reported following frontal cortex lesions in rats and primates correspond to those reported in man (Kolb, 1984). Additionally, learning tasks performed in rats following medial frontal cortex lesions indicate poor performance in motor learning (Kolb & Whishaw, 1983), spatial learning (Kolb et al., 1983), and second-order conditioning procedures (Kolb et al., 1974; McDonough & Manning, 1979). Acquisition of classical aversive conditioning, however, was not affected (Kolb & Whishaw, 1981).

Recent anatomical studies of the medial frontal cortex in the rat have shown direct connections with autonomic centers in the brainstem (van der Kooy, 1982; Terreberry & Neafsey, 1983, 1987) and the intermediolateral cell column of the

spinal cord (Hurley-Gius et al., 1986). Stimulation of the ventral portion of the rat medial frontal cortex produces prominent cardiovascular, respiratory and gastric changes (Kaada, 1960; Lofving, 1961; Terreberry & Neafsey, 1984; Hurley-Gius & Neafsey, 1986; Al Maskati & Zybroyna, 1989). Lesions of the medial frontal cortex of the rat alter cardiovascular responses to stress (Szilagyi, 1987) and affect the gain of the cardiac component of the baroreceptor reflex (Verberne et al., 1987). These studies indicate that the medial frontal cortex may play an integral role in cardiovascular and other visceral responses.

Experimental and clinical evidence indicates that the telencephalic limbic structures are centrally involved in functions related to the animal's internal milieu (Nauta, 1971), and the frontal cortex appears to be a major monitor and modulator of the limbic system. Nauta predicted that lesions of the frontal cortex could be expected to greatly affect the animal's "viscero-endocrine, affective and motivational responses to his environment." He also suggested that autonomic and/or behavioral responses to a variety of events, including stress, would be permanently altered by frontal lesions.

One paradigm for studying stress-related behavioral and autonomic changes is the conditioned emotional response (Smith et al., 1980; Turrkhan, 1989; Cohen & Randall, 1984; Iwata & LeDoux, 1988). This paradigm involves classical conditioning of a neutral stimulus, such as a tone, with a stressful or aversive stimulus, after which the neutral stimulus alone evokes the same response as the aversive stimulus. In the unrestrained rat, the cardiovascular conditioned response

is increased blood pressure and heart rate to the conditioned stimulus. Recent studies on baroreceptor activation during stress indicates a significant reduction in the cardiac baroreflex gain during aversive conditions, and therefore may account for the unusual pairing of increased blood pressure and heart rate during stress. The relative contributions of sympathetic and parasympathetic influences is also greatly affected by stress, and therefore must also be included in any study of conditioned emotional responses. Respiratory and behavioral responses in relation to stress and conditioned emotional responses are also known to be altered, but previous behavioral studies of cardiovascular responses to stress have generally ignored respiration (Grossman, 1983). Respiration is a powerful modulator of cardiovascular activity via both the parasympathetic (Katona et al., 1970) and sympathetic (Cohen & Gootman, 1970) nervous systems. Behavior associated with stress includes freezing, ultrasonic vocalization, defecation and other species specific behaviors, all or some of which may be modulated by cortical areas.

According to Powell et al. (1989), "structures rostral to the hypothalamus, probably in the amygdala and/or agranular prefrontal cortex, mediate the (cardiovascular changes) associated with classical conditioning contingencies." Lesions of the medial frontal cortex result in changes in behavior, cardiovascular responses to stress, and the resting baroreflex gain. The purpose of this dissertation project, therefore, was to determine the role of the ventral medial frontal cortex in the awake rat on cardiovascular, autonomic, respiratory and behavioral (freezing and ultrasonic vocalization) conditioned emotional responses.

CHAPTER II

REVIEW OF RELATED LITERATURE

STRESS AND THE CARDIOVASCULAR SYSTEM

Cardiovascular disorders are major health problems (Cohen & Cabot, 1979), and a significant factor in their etiology appears to be emotional stress (Lawler et al., 1980, 1981; Obrist, 1981; Verrier & Lown, 1984; Talman, 1985; Henry et al., 1986). A human or animal has two means of coping with situations perceived as stressful or life-threatening. One response is to actively cope (fight); the alternative is to passively cope (submit). The cardiovascular responses associated with each type of response are markedly different. Active coping evokes excitation of cardiovascular activity (increased blood pressure, heart rate and cardiac output), while submission generally evokes an inhibition of myocardial activity (Obrist, 1981). While these forms of response are appropriate for animals in the wild, due to our socialization they are no longer appropriate for us much of the time, resulting in pathological consequences. As Folkow & Neill reported in 1971:

> "A civilized man, whose defense reaction (a cardiovascular response similar to beta-adrenergic mediated effects) is aroused by repeated arguments with his boss, reluctantly suppresses the somatomotor component and avoids both flight and fight. The repeated autonomic-hormonal-metabolic mobilization thus occurs largely 'in vain,' as no rapid burn-off occurs, much against nature's intentions. It is possible that such dissociated patterns, often repeated over years, may lead to pathophysiological states."

It is also known now that these cardiovascular responses can be "learned" or conditioned to various stressful events, thus allowing cardiovascular responses to occur in anticipation of certain events and/or behaviors, regardless of whether the event actually occurs.

EXPERIMENTAL MODELS OF EMOTIONAL STRESS

A particularly effective model for studying the relationship between behavior and cardiovascular activity is classical (Pavlovian) conditioning. During classical conditioning a stimulus (S1) is dependent upon the occurrence of another event (S2). In this context, S1 is generally labeled as the unconditioned stimulus (US) and S2 as the conditioned stimulus (CS). Some relation is arranged between S1 and S2 during the acquisition phase (T1) without regard to the animals behavior. The consequences of that relation are then assessed at some other time (T2). If the arrangement of the relation between S1 and S2 can be identified as being responsible for the behavior seen at T2, then classical conditioning can be said to have occurred (Rescorla & Holland, 1974). The classical conditioning paradigm is often described as selecting a US (such as a shock) that reliably evokes a response and pairing it with a "neutral" CS (such as a tone) until the CS alone evokes the response.

Classical conditioning is not the only method for producing learned cardiovascular responses. Other paradigms which present external stimuli in order

to produce a response from an animal include (but are not limited to) the startle paradigm (often associated with habituation) and instrumental conditioning paradigms (Rescorla & Holland, 1974).

Startle

The startle response paradigm of behavioral study is the simplest and is often used in conjunction with habituation studies. The question addressed by this paradigm is whether simple exposure to a stimulus at T1 produces differential behavior to the same stimulus at T2; assessment occurs entirely at T2 compared to the response seen at T1. This paradigm allows for easy interpretation of the variables studied, but severely limits the types of observations which can be made, due to the variability caused by random events between testing conditions.

Presentation of a loud noise (S) at T1 typically reduces the likelihood that this noise will evoke a startle response at T2 (Caeser et al., 1984). Habituation is generally considered to account for this type of change. The term habituation, however, is not utilized uniformly by all investigators. Some use the term in reference to the outcome (decrement of the response), while others refer to it as a procedure (repeated stimulus presentation). The use of repeated stimulus presentation results in a typical reduction in the response seen, and can be plotted over the number of trials given. This procedure greatly reduces the effects of "random" events mistakenly incorporated into the response, since a random event would typically result in a deviation from the normal, projected habituation curve.

Substantial literature has been generated in this field, as is evidenced in the reviews by Davis & File, 1984, Hoffman & Ison, 1980, Groves & Thompson, 1970 and Hinde, 1970. Startle responses, however, are generally considered as "reflexive", and are not thought of as associative learning.

Instrumental Conditioning

This paradigm uses a particular response or behavior by the animal to signal the presentation of the unconditioned stimulus. The question at issue in this form of conditioning is whether the establishment of a relation between the occurrence of a particular (and often learned) behavior and that of a stimulus produces differential behavior at T2 (Rescorla & Holland, 1974). It is obvious that the number of variables in this form of conditioning is extremely high, and comparisons between different procedures are difficult. Differences in procedures can include the behavior used to signal the stimulus (bar press, avoidance, etc.), the stimulus used (aversive, appetitive, reward, etc.), the variables normally encountered in classical conditioning (stimulus intensity, intertrial interval, etc.) and variables intrinsic to each animal (ability to perform the behavior, lesions, etc.). Each of these variables in this form of behavioral learning are no longer independent, but rather tend to be embedded within one another (Rescorla & Holland, 1974; Macintosh, 1981). These factors make instrumental conditioning highly complex, and according to Rescorla & Holland (1974) it may be "a poor paradigm for initial study of the biology of learning."

Classical Conditioning

Classical conditioning, on the other hand, directly relates a response to a particular stimulus. Many factors influence the magnitude of the conditioned response (CR), such as the number of pairings administered, the interval between the CS and US, the strength and/or duration of the US and the use of appropriate controls (Rescorla & Wagner, 1972). The advantages of using this paradigm are numerous, and include: 1) precise stimulus control; 2) stimulus- locked cardiovascular responses; 3) rapid establishment of reliable responses; 4) the ability to use instrumented, awake animals; 5) a large body of literature on the relevant behavioral and anatomical control variables (Cohen & Randall, 1984). Of particular concern to most investigators interested in studies utilizing classical conditioning are the use of proper controls, and how to ensure that the response at T2 was "conditioned."

The use of a separate group which did not receive the aversive US was the earliest form of control. However, these "control" groups which did not receive the aversive stimulus were, in a sense, notified by the conditioned stimulus that no aversive event would occur, and therefore were negatively conditioned. This was viewed by many as a false control. Many variations and combinations were proposed to provide a true control (see review in Rescorla & Holland, 1974), until Rescorla (1967) provided the first "random control" procedure involving presentation of a second conditioning stimulus termed the CSr. The CSr eliminated the statistical relationship between the CS and US, so that the occurrence of the US

was unpredictable; the US could occur before, during, after, or not at all in relation to the CSr. The CS which was directly related to the US (CS consistently paired with the US) was then termed the CS+. Use of distinct CS+ and CSr stimuli resulted in a learning paradigm which could differentiate the conditioned response (CR) to the CS+ from the normal orienting response to the CSr, which was randomly related to the US.

Conditioned Cardiovascular Responses

Use of classical conditioning to investigate stress induced cardiovascular responses was introduced by Smith (1967), who termed the paradigm the conditioned emotional response (CER). The CER is now commonly employed in many cardiovascular studies (see reviews by Cohen & Randall, 1984; Turkkhan et al., 1982; Iwata & LeDoux, 1988). When a CS+ is repeatedly paired with a shock (US), the subsequent CS+ only presentation produces increases in blood pressure and heart rate (LeDoux et al., 1984; see review in Iwata & LeDoux, 1988), increased myocardial contractility (Randall & Smith, 1974), increased aortic and renal blood flow (Smith et al., 1979), increased cerebral blood flow and cardiac output (LeDoux et al., 1983), and increases in total peripheral resistance (Randall et al., 1982). The nature of the response varies considerably with the species of the animal used, the nature of the US, and the emotional state of the animal. Rabbits and restrained rats show a depressor response linked with a bradycardia (Buchanan et al., 1985; Powell et al., 1985, 1989; Buchanan & Powell, 1982; Francis et al., 1981;

Iwata & LeDoux, 1988), while unrestrained rats and primates respond to the CS with a pressor response and a tachycardia (Smith et al., 1980; Martin & Fitzgerald, 1980; Iwata & LeDoux, 1988).

The effects of lesions of a variety of brain areas on the CER have been studied. For example, lesions of the auditory system, such as the medial geniculate, resulted in the elimination of the conditioned emotional responses to auditory stimuli (LeDoux et al., 1984; Ledoux et al., 1986; Jarrell et al., 1986, 1987; Teich et al., 1989). Lesions of the central nucleus of the amygdala also resulted in attenuation or complete loss of various conditioned responses (Hitchcock & Davis, 1987; Gentile et al., 1986a & b; Kapp et al., 1979; Vanderwolf et al., 1988). Lesions of the hypothalamus resulted in loss of mediation of portions of the learned cardiovascular responses (Iwata et al., 1986; Powell & Levine-Bryce, 1989). Projections of the central nucleus of the amygdala (central gray and lateral hypothalamus) independently mediate the behavioral (freezing) and autonomic (blood pressure) correlates (respectively) of conditioned fear (LeDoux et al., 1988). Insular cortex lesions in the rabbit did not affect the conditioned bradycardia (Powell et al., 1985), whereas medial prefrontal cortex lesions resulted in attenuation of the conditioned bradycardia (Buchanan & Powell, 1982). Table 1 summarizes briefly the effects of various lesions on the CER. Note that no CER studies, however, have been performed following lesions of the more ventral, infralimbic region of the medial frontal cortex in the rat.

MEDIAL FRONTAL CORTEX

The rat medial frontal cortex (MFC), located on the medial surface of the cerebral hemispheres, extends from the genu of the corpus callosum to the frontal pole. This cortical area has been subdivided cytoarchitecturally by Krettek & Price (1977) and Vogt & Peters (1981) from dorsal to ventral into anterior cingulate, prelimbic, and infralimbic cortices; the taenia tecta marks the ventral boundry of the medial frontal cortex (see Figure 1).

The infralimbic and prelimbic regions of the medial frontal cortex send direct projections to the solitary nucleus and thoracic intermediolateral cell column (Saper, 1982; Terreberry & Neafsey, 1984, 1987; van der Kooy et al., 1984; Hurley-Gius et al., 1986). The projections to the solitary nucleus are important, since this is the primary site for integration of many visceral and autonomic afferents. Projections course from the medial frontal cortex through the pyramidal tracts to their decussation in the medulla. Here, the fibers leave the pyramidal tract and synapse bilaterally in the various subnuclei of the NTS (Terrebery & Neafsey, 1987; van der Kooy, 1984). Termination of these fibers occurs in the ventral, ventrolateral, and intermediate subnuclei, with light to moderate labeling in the dorsolateral, dorsal, medial, commissural and interstitial subnuclei of the NTS (Terreberry & Neafsey, 1987; van der Kooy, 1984).

The MFC also has connections with the insular cortex (Saper, 1982; Markowitsch & Guldin, 1983), mediodorsal nucleus of the thalamus (Domesick, 1969, 1972; Leonard, 1969; Beckstead, 1976; Krettek & Price, 1977), amygdala

(Krettek & Price, 1977; Ricardo & Koh, 1978; Price, 1981), hippocampus (Swanson, 1981; Ferino et al., 1987; Ruit & Neafsey, 1988), lateral dorsal tegmental nucleus (Terreberry & Neafsey, 1987) and the ventral tegmental area (Lindvall et al., 1974, 1978), as well as contralateral, homotopic cortex (Domesick, 1969; Beckstead, 1979; Ferino et al., 1987). In addition, a large efferent projection terminates in the central grey (PAG) and the superior colliculus (Hardy & Leichnetz, 1981; Wyss & Sripandkulchai, 1984; Neafsey et al., 1986). Figure 2 summarizes the connections of the medial frontal cortex.

Electrical or chemical stimulation of the medial frontal cortex produces prominent changes in heart rate, blood pressure, respiration and gastric motility (Burns & Wyss, 1985; Hurley-Gius & Neafsey, 1986; Kaada, 1960; Lofving, 1961; Terreberry & Neafsey, 1984; Buchanan et al., 1985; Hardy & Holmes, 1988). Al Maskati (1989) has classified electrical and chemical stimulation sites within the dorsal MFC (corresponding to the anterior cingulate and prelimbic regions) as "sympatho-inhibitory." Stimulation of these areas in anesthetized or awake rats results in attenuation or inhibition of the defense response and the associated cardiovascular components, while stimulation of the ventral MFC results in an additive response between the depressor/bradycardia elicited by the ventral MFC and the pressor/tachycardia elicited by the defense response. In anesthetized rats, stimulation of the ventral MFC results in a distinct bradycardia, typically associated with a depressor response and a decrease in respiratory rate and volume (Terreberry & Neafsey, 1986).

Lesions of the medial frontal cortex result in impairment of spatially oriented learning behaviors (Kolb, 1984), increases in spontaneous motor activity (Silva et al., 1986; Sinnamon & Charman, 1988; Ferino et al., 1987; see also review by Kolb, 1984), and cardiovascular changes, including impairment of habituation (Szilagyi et al., 1985; Skinner & Reed, 1981), reduced hypertension, decreased baroreceptor reflex gain (Verberne et al., 1987) and attenuation of the blood pressure and heart rate responses (Buchanan & Powell, 1982 a, b).

Studies by Thierry et al. (1976) show a highly specific increase in DOPAC levels and dopamine turnover in the MFC during stress, and 6-hydroxy-dopamine lesions of the frontal cortex result in attenuation of hypertension in spontaneously hypertensive rats (Van den Buuse et al., 1986). Table 2 summarizes some of the cardiovascular and behavioral responses associated with the medial frontal cortex. It is apparent from this brief summary that the medial frontal cortex is intimately associated with many limbic and behavioral processes, and may play a key role in the modulation of these processes.

Stress and Respiratory Related Responses

Many previous studies of cardiovascular responses to stress have ignored respiration (Grossman, 1983), even though variations in breathing parameters can modulate both tonic and phasic cardiovascular responses (Sheperd, 1981; Hirsch & Bishop, 1981). Variations in breathing patterns are greatly influenced by behavioral, emotional and cortical factors (Phillipson, 1978; Suess et al., 1980;

Fokkema et al., 1986). Respiratory patterns associated with stress can result in dysfunctional cardiovascular alterations, such as reduced parasympathetic cardiac control (Hirsch & Bishop, 1981; Katona & Jih, 1978) and inadequate cerebral oxygenation (Case & Greenberg, 1976).

Psychophysiological studies of cardiac responses tend to emphasize beta-adrenergic effects, ignoring parasympathetic influences and sympathetic-parasympathetic interactions (Grossman & Svebak, 1987). However, recent evidence indicates that within- and between-animal variations in cardiac parasympathetic heart rate control can be accurately indexed by measurement of respiratory sinus arrythmia (RSA), with fluctuations in cardiac interbeat interval corresponding to the phase of respiration (Akselrod et al., 1981; Katona & Jih, 1975; McCabe et al., 1985; Porges, 1986; Grossman & Svebak, 1987). At rest, sinus arrhythmia is in phase with respiration (Marshall, 1961): heart rate increases during inspiration and decreases during expiration (Marshall, 1961; Angelone & Coulter, 1964). Measurement of RSA assesses, on a breath-by-breath basis, the shortest R-R heart rate interval corresponding to inspiration and the longest R-R interval corresponding to expiration. The magnitude of RSA is the difference (in msec) between the "peak" HR response measured during inspiration and the "trough" HR response measured during expiration (for a detailed description of the procedure used in this study, see Chapter 8). Since the amount of parasympathetic control is directly related to the decrease in HR period, RSA analysis is a strong, non-invasive measurement of parasympathetic activity in both awake and anesthetized animals

(Grossman & Svebak, 1987; Katona & Jih, 1975; Fouad et al., 1984; McCabe et al., 1985).

The classic Valsalva maneuver is a good example of the direct connection between respiratory (increased intrathoracic pressure) and cardiovascular (increased blood pressure) components. A similar response occurs naturally in freely behaving rats in a social "defeat" situation. Fokkema et al. (1986) described a large amplitude blood pressure oscillation corresponding to respiratory movements during defeat in a territorial fight between male rats, and subsequently during a psychosocial stimulus associated with the defeat (presentation of the dominant rat without physical contact). The blood pressure oscillation coincided with the respiratory pattern known as "pressure breathing," during which increased intrathoracic pressure is associated with expiration against a closed glottis. The expirations were prolonged and accompanied by increased blood pressure and blood pressure variability, decreases in heart rate, freezing, and occasionally ultrasonic vocalizations (USV). The length of expression of these parameters often outlasted the presentation of the stimulus.

Stress and Ultrasonic Vocalizations

Ultrasonic vocalizations are common in many rodent species, and most both produce and hear sounds in the ultrasonic range (Nyby & Whitney, 1978). Ultrasounds apparently play an important role in intraspecies communication, and have been implicated in many behavioral contexts, such as parent-offspring

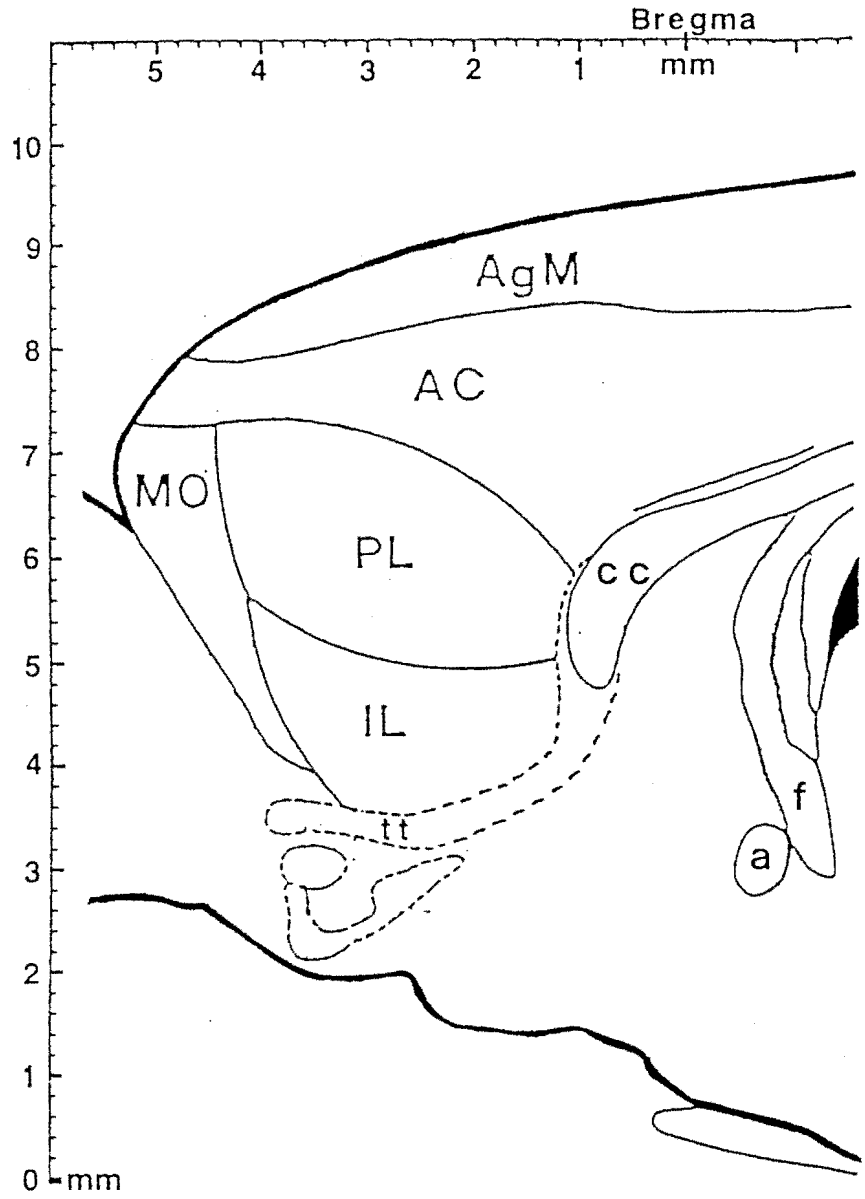
communication, aggression, courtship and mating behaviors, territoriality, and stress. Much of the work has centered around infant-mother calling, and this literature has been extensively reviewed by Noirot (1972), Sales & Pye (1974) and Brown (1976).

Ultrasonic vocalizations in adult rodents show considerable intra- and interspecies variation in frequency. Two types of USV have been identified: a long call and a short call. The long call frequency ranges from 22 to 30 kHz with a duration of 400 to 1600 msec; the short call frequency ranges from 40 to 75 kHz with a duration of 3 to 200 msec (Sales, 1972, 1974; Brown, 1973, 1976; Ghiselli & LaRiviere, 1977). The long call occurs simultaneously with flank (respiratory) movements accompanying exhalation (Roberts, 1972), and there is typically only one vocalization per expiration. Following social aggression encounters, the defeated rat assumes a submissive crouch posture (freezing behavior) and produces long call ultrasounds of relatively constant frequency, ranging from 23 to 30 kHz (Corrigan et al., 1977; Nyby & Whitney, 1978; Fokkema et al., 1986; Berg & Baeninger, 1973; Sales, 1972). Long calls have also been detected following other behavioral stressors, such as handling (Sales, 1979), shock-elicited aggression (Ghiselli & LaRiviere, 1977; Tonoue et al., 1986) and during the post-ejaculatory phase in male rats (Barfield & Geyer, 1975). Ultrasound is severely disrupted when either the superior or inferior laryngeal nerves are cut, suggesting that the site of ultrasound production is within the larynx (Roberts, 1975). There have not been any studies to date which have examined respiration and ultrasonic vocalizations

during the CER.

As mentioned previously, the medial frontal cortex has a large projection to the central gray, a structure associated with fear (Irisawa & Iwasaki, 1982; Wada et al., 1970), rage (Bandler, 1982) and other defensive and emotional behaviors (DiScala et al., 1987); central gray stimulation has been used as an aversive stimulus (Irisawa & Iwasaki, 1982; DiScala et al., 1987). Vocalizations have been recorded by stimulation of the central gray in many species, including the rat, cat and monkey (Jurgens & Pratt, 1979; Kanai & Wang, 1962; Larson & Kistler, 1984; Larson, 1985). Lesions of the central gray have resulted in loss of vocalizations (Adametz & O'Leary, 1959; Jurgens & Pratt, 1979). Recently, lesions of the midline frontolimbic cortex in the squirrel monkey have shown a loss of vocalization following complete lesions of the frontal "limbic cortex" (Aitken, 1981; Sutton & Larson, 1986; Newman & MacLean, 1985; MacLean & Newman, 1988), and that incomplete or lesser lesions do not result in this loss. MacLean and Newman postulated that the anatomical connections (medial frontal cortex to central gray; central gray to nucleus ambiguus) suggest that the medial frontal cortex may be implicated in both motivation and the expression of vocalizations, specifically the separation call.

Figure 1. Sagittal view of the rat medial frontal cortex 0.4 mm lateral to the midline displaying the cytoarchitectural boundries used in this study. AC, anterior cingulate cortex; AgM, agranular medial cortex; CC, corpus callosum; IL, infralimbic cortex; MO, medial olfactory cortex; PL, prelimbic cortex; a, anterior commissure; f, fornix; tt, taenia tecta.



Lateral 0.4mm

Figure 2. Illustration of medial frontal cortex connections with autonomic and behavioral centers. Arrows denote direction of projections. CeA, Central Nucleus of the Amygdala; Bl, Basolateral Nucleus of the Amygdala; L, Lateral Nucleus of the Hypothalamus; Dl, Dorsolateral Nucleus of the Hypothalamus; Re, Reuniens Nucleus of the Thalamus; Pt, Parataenial Nucleus of the Thalamus.

ANATOMICAL CONNECTIONS OF THE RAT MEDIAL FRONTAL CORTEX

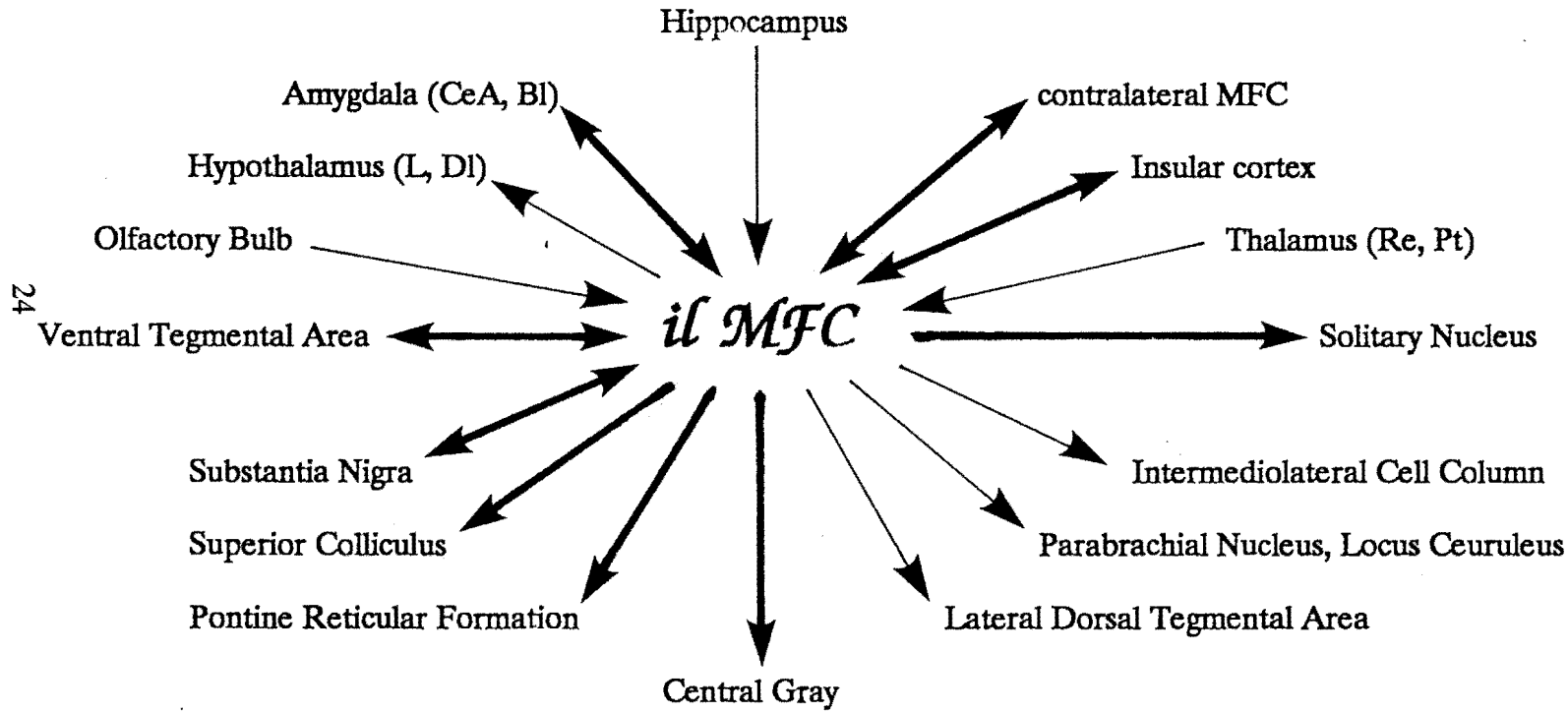


Table 1. **BRAIN LESION EFFECTS ON CONDITIONED RESPONSES**

Site of Lesion	Effect of Lesion	Reference
Central Nucleus Amygdala	Impaired startle response (rat)	Hitchcock, 87
	Impaired conditioned defecation (rat)	Vanderwolf, 88
	Abolish HR conditioned response (rabbit)	Gentile, 86
	Decrease cardiovascular noise response (rat)	Galeno, 84
	Attenuation of conditioned bradycardia (rabbit)	Kapp, 79
Lateral Hypothalamus	Abolish BP CER; exploration intact (rat)	Iwata, 86
	Abolish conditioned bradycardia (rabbit)	Powell, 89
	Decrease conditioned bradycardia (rabbit)	Francis, 80
	Abolish CV CR; behaviors intact (baboon)	Smith, 80
Central Gray	Disrupt freezing; cardiovascular CER intact (rat)	LeDoux, 88
Lateral Subthalamic N	Abolish conditioned bradycardia (rabbit)	Jarrell, 86
Substantia Nigra	Decrease conditioned eyeblink (rabbit)	Kao, 86
Medial Geniculate N	Suppressed cardiovascular & freezing CER (rat)	LeDoux, 83
	Attenuation of conditioned bradycardia (rabbit)	Jarrell, 87
Anterior Cingulate Cx	Prevent conditioned HR extinction (rabbit)	Teich, 89
	No CER changes noted (rat)	LeDoux, 83
	Medial abolished HR CER; Lateral no response (rat)	Buchanan, 82
Insular Cx	Attenuation of conditioned bradycardia (rabbit)	Powell, 85

Table 2. **FUNCTIONS OF THE MEDIAL FRONTAL CORTEX**

Behavior/Response	Nature of Response Following MFC Lesion	Reference
Response Inhibition	Impaired performance on tasks requiring changing behavior	Kolb, 1974
Spatial Orientation	Poor spatial learning	Kolb, 1983
Social Behavior	Increased fighting; homosexual behavior	Lubar, 1973; Kolb, 1974
Habituation	Head poke prevented/retarded;	Glaser, 1962; Butter, 1964; Kolb, 1974
Contralateral Neglect	No response to sensory stimuli	Crown, 1982
Food Hoarding	Significantly decreased	Stamm, 1954; Kolb, 1974; Whishaw, 1989
Maternal Behaviors	Poor pup retrieval; Nest building impaired	Stamm, 1955; Wilsoncroft, 1963; Shipley,1977
General Activity	Typically increased	Richter, 1939; Kolb, 1974
Cardiovascular CER	Baroreflex reduced; Hypertension reversed	Verberne, 1987; Szilagyi, 1987
Vocalizations	- - Unknown - -	
Freezing Behavior	- - Unknown - -	

CHAPTER III

OBJECTIVES AND HYPOTHESES

OBJECTIVES

The main objective of my dissertation research was to determine to what extent the MFC influences cardiovascular, respiratory, and ultrasonic vocalization responses during the CER in the awake, behaving rat, and through what mechanisms (modulation of baroreflex gain, "direct" activation of parasympathetic & sympathetic nervous systems, etc.) this occurred.

HYPOTHESES

The four primary hypotheses listed below were tested. "CS" responses are defined as those responses which occur during the CS (seconds 21 through 31). "Late" responses are defined as the responses beginning 10 seconds after the cessation of the CS through the remaining time in the CER trial (seconds 40 through 108).

HYPOTHESIS 1. The normal cardiovascular, respiratory and ultrasonic vocalization pattern to a CS is influenced by the MFC.

To test this hypothesis, the normal CER pattern was determined and then compared to the CER pattern of MFC lesioned animals.

HYPOTHESIS 2. During the stress response to a CS, the MFC modulates the gain of the baroreceptor reflex, producing the cardiovascular responses seen in the normal, unoperated rat (increased blood pressure and heart rate).

To test this hypothesis, several questions needed to be answered. First, what is the contribution of the baroreceptor afferents to the normal response pattern? This was determined by comparing the CER response in control animals with animals having sustained SAD lesions. Second, what is the contribution of the MFC to the baroreflex gain changes seen during the CER? This was determined by comparing baroreflex gain changes during the various phases of the CER (baseline, during the CS, 10 sec after the CS, and 1 min after the CS) in control animals with animals having MFC lesions. Third, what is the contribution of the MFC independent of baroreceptor afferents? This was determined by comparing SAD lesioned animals with those animals that had both SAD and MFC lesions. Fourth, what are the residual CER responses in animals with both SAD and MFC lesions? This was determined by comparing responses from Controls with responses from SAD + MFC lesioned animals. That portion of the response not due to either baroreceptor afferents or MFC influence would be the residual response, presumably due to the action of other brain regions such as the amygdala or hypothalamus.

HYPOTHESIS 3. The MFC is altering sympathetic and/or parasympathetic outflow directly during the cardiovascular CER.

Through the use of appropriate pharmacological blocking agents, we attempted to answer the following questions. First, to what extent is the normal (control) CER response mediated by parasympathetic influences? Methyl atropine, a muscarinic cholinergic blocker, was used to eliminate vagal parasympathetic influences. By comparing responses before and after methyl atropine administration, the component of the response that is mediated by parasympathetic influences would be eliminated, with the remaining portion of the response being due to sympathetic influences. Second, to what extent is the normal CER response mediated by beta-adrenergic components of the sympathetic system? Atenolol, a cardio-selective beta adrenergic blocker, was used to eliminate myocardial beta-adrenergic sympathetic influences, with the residual response being due to parasympathetic and sympathetic alpha-adrenergic influences. Finally, the sympathetic and parasympathetic components were simultaneously blocked by the combination of methyl atropine and atenolol. Similar tests were performed in the MFC lesioned group to determine any changes in the vagal and sympathetic components of the CER in MFC lesioned animals.

HYPOTHESIS 4. During the late portions of the CER, an increase in respiratory sinus arrythmia (RSA) related to respiratory modulations during USV will be found.

In order to test this hypothesis, the amplitude and period of respiratory sinus arrythmia (RSA) during the late portion of the CER was analyzed. Several questions needed to be answered. First, what is the normal RSA amplitude and period during rest? This was determined by analyzing a 2 minute baseline trial recorded prior to CER testing. Second, what is the normal RSA amplitude and period during the CER? This was determined by quantifying RSA in Controls during the CER. Third, what influences does the MFC have on RSA amplitude and period during the CER? This was determined by comparing the RSA values obtained from Control and MFC lesioned animals.

CHAPTER IV

GENERAL METHODS

The information needed to investigate the BP, HR, respiration rate and USV response patterns and characterize the role of the MFC in relation to stress was obtained through a study of four experimental groups. Male Sprague-Dawley rats weighing 250-500 g were used throughout the experiment.

GROUP 1: Intact baroreceptor afferents + Sham MFC lesion (N = 13). This group defined the normal response patterns during the CER and baroreflex tests; in addition, it provided baseline (control) values for comparisons between groups.

GROUP 2: Intact baroreceptor afferents + MFC lesion (N = 11). This group defined the effects of MFC lesions on the CER responses, as well as the effect of MFC lesions on the baroreflex.

GROUP 3: Sinoaortic denervation + Sham MFC lesion (N = 5). This group defined the extent of involvement of the baroreceptor afferents during normal CER response patterns and also provided baseline values for comparisons with Group 4.

GROUP 4: Sinoaortic denervation + MFC lesion (N = 4). This group defined the involvement of the MFC on the CER response patterns in the absence of the baroreflex.

In all groups, the basic CER response pattern for each parameter was determined. Alterations in the gain of the baroreceptor reflex during the CER were measured by activating the reflex through brief (2 sec) inflations of an aortic balloon catheter during various phases of the CER in groups 1 and 2 only. In SAD lesioned rats, this portion of the testing was omitted, since denervation eliminated the HR response to BP fluctuations. Assessment of the contributions of the sympathetic and parasympathetic nervous systems in producing the CER pattern were then tested through selective pharmacological blockade in groups 1 and 2 only. Finally, mathematical "peak-trough" analysis of the relationship between heart period and respiration was performed on the data collected during the resting (baseline) and CER trials to determine the period and amplitude of RSA during the CER.

Control for Orienting Response:

As a control for the conditioning paradigm used, CSr responses were recorded and compared to CS+ responses within each group to determine whether orienting reflexes to a tone randomly paired with footshock (nonassociative response) played a significant role in the response patterns generated.

CHRONOLOGY OF PROCEDURES

The chronological order of the procedures each rat was subjected to was:

1. SAD lesion/sham - 2 week recovery period
2. MFC lesion/sham - 2 week recovery period
3. Classical conditioning of animal, followed by implantation of cardiovascular and respiratory recording hardware - 24 hour recovery
4. Recording of cardiovascular, respiratory, and ultrasonic vocalization CERs
5. Sacrifice by anesthetic overdose
6. Data analysis

Detailed descriptions of the procedures common to all animals in this study are discussed below. Procedures specific to the various portions of this study are discussed individually in the appropriate chapters.

Conditioning Parameters

During conditioning, each animal was placed in a clear, lighted plexiglass box with a metal grid floor (2 mm diameter metal rods, spaced 1 cm apart) and an overhead speaker. A high frequency, ultrasonic microphone was also placed under the grid floor in order to record ultrasonic vocalizations during conditioning. The output of the microphone was displayed on an oscilloscope. The entire conditioning apparatus was then insulated from outside noise by enclosing it in a sound resistant box. Conditioning included both a CS+ tone (950 Hz) that was consistently paired with a footshock and a CSr tone (300 Hz) that was randomly paired with footshock (Rescorla, 1967). The two tones (950 or 300 Hz) were presented for 10 seconds at 80 db at random intervals (10 sec to 5 min) through the overhead speaker. Forty tones (averaging 20 tones each for CS+ and CSr) were presented, with the order of presentation randomly chosen by the computer. The CS+ tone was immediately followed by a 0.5 second, 2 milliamp footshock (US) delivered through the grid floor. The footshock produced a strong tingling sensation to my hand which was uncomfortable, but did not cause "pain" or tissue damage. The CSr tone was randomly associated with the same footshock, meaning that the footshock occurred before, during, or after the tone, or not at all. All functions of the conditioning session were controlled by the MINC 11/23 laboratory computer, which determined the frequency, inter-trial interval, and order of CS+/CSr presentation using a

random number generator. During conditioning, vocalization was monitored on an oscilloscope.

Cannula Implantation

Immediately following conditioning, arterial and venous cannulas were implanted in order to monitor arterial blood pressure and introduce intravenous drugs. Additionally, an arterial balloon catheter was implanted in order to produce transient rises in blood pressure elicit the gain of the baroreflex. The animal was anesthetized with ketamine HCl (100 mg/kg) and restrained in a supine position on a rat board. Electrocardiogram (ECG)/ respiration (RESP) leads, composed of teflon coated, multi-stranded stainless steel wires fitted with amphenol connectors, were then implanted in the lateral aspects of the thoracic cage (at the points of maximal change during inspiration) through small stab wounds. The free ends were tunnelled subcutaneously to the base of the neck and exteriorized through a puncture wound. The ventral hindlimb thigh was incised, and the femoral artery and vein carefully dissected free from the neurovascular sheath and surrounding fat. The artery was clamped and incised, and a saline-filled, 2F Fogarty balloon catheter threaded inside the vessel to the level of the thoracic aorta (approximately 10 cm). The free end of the catheter was attached to 0.04" diameter Microrenathane tubing. The vein was cannulated with a polyethylene tube (PE50) threaded inside the vessel to the level of the inferior vena cava (approximately 5 cm). After determining the patency of the venous line, it was flushed with heparinized saline (50 U/ml) and

heat sealed to prevent leakage. The free end of each catheter was tunnelled subcutaneously to the base of the neck, exteriorized through the puncture wound previously made for the ECG and RESP leads, and marked for future reference. The wound in the leg was sutured and swabbed with bactericidal soap.

Two approaches were used to record arterial pressure. One approach utilized the brachial artery, the other utilized the femoral artery opposite the balloon catheter. In the brachial artery approach, the ventral aspect of the forelimb in the axillary region was incised, and the brachial artery dissected free from the neurovascular sheath. The artery was cannulated with PE10 tubing to the point of origin of collaterals (approximately 2 cm). The free end of the PE10 tubing was mated to PE50 in order to facilitate connection to the transducer during recording. After determining patency of the catheter, it was flushed with heparinized saline (100 U/ml) and heat sealed to prevent leakage. The free end of the catheter was tunneled subcutaneously to the nape of the neck and exteriorized. The wounds were sutured, swabbed with bactericidal soap and the animal returned to his home cage to recover for 24 hours.

In the alternate femoral artery approach, the ventral aspect of the hindlimb opposite the balloon catheter was incised, and the femoral artery carefully dissected from the neurovascular sheath. The artery was clamped and polyethylene tubing (PE10) was threaded inside the vessel to a level approximately 1 cm ABOVE that of the balloon (approximately 11 cm). PE10 did not impede circulation in the femoral artery due to its relatively small diameter. The free end of the PE10 was

mated to PE50 in order to facilitate connection to the transducer during recording. After determining patency of the catheter and collateral circulation around it, the catheter was flushed with heparinized saline (100 U/ml) and heat sealed to prevent leakage. The free end was tunneled subcutaneously to the nape of the neck, exteriorized, and marked. Any animal that showed serious side effects of either surgical approach (such as limb disuse or abnormal gait) was sacrificed as soon as possible using anesthetic overdose with sodium pentobarbital (300 mg/kg). In most cases, the rats had full use of all limbs the following day. No significant differences were seen between the two arterial cannulation approaches; the brachial artery approach was generally used only if the ventral hindlimb approach failed, or was contraindicated (small arteries, poor collateral circulation, etc.)

Data Acquisition

On the day following conditioning, each animal was brought to the recording room in his own cage where he remained. The catheters, ECG and RESP leads were connected; 30 minutes were allowed to pass before any CS were presented to allow the animal to return to a "resting" state. Blood pressure, heart rate, respiration, and ultrasonic vocalization were continuously recorded on a polygraph; in addition, during the actual CER trials, these four parameters were input to the computer's digital or analog ports for storage and analysis. Computer sampling of all four parameters took place at the occurrence of each R wave in the ECG signal (approximately 5 Hz). Heart rate (bpm) was derived by detecting R waves in the

ECG signal (recorded from electrodes in the lateral chest wall musculature) with a window discriminator; the window pulse output was sent to a rate meter which was connected to the polygraph. During CER trials, the window output also provided a digital signal to the computer which then stored heart rate values calculated directly by computing the reciprocal of the interval since the last R wave. Blood pressure was recorded via the arterial catheter attached to a Statham blood pressure transducer. Data sampled at the R-wave of the ECG signal corresponded to approximately 50% of the systolic/diastolic range in pressure, as illustrated in figure 3. Output of the signal was put through a low pass filter in order to obtain mean arterial pressure. Respiration was monitored by measuring the transthoracic impedance between chest electrodes with a UFI model 2991 impedance converter. Output of the signal was sent continuously to the polygraph, and sent to the computer at the occurrence of the R-wave of the ECG signal (approximately 5 hz). Ultrasonic vocalizations were monitored on an oscilloscope via the output of a high frequency Panasonic condenser microphone (PF9932; sensitive to 75 kHz). The signal was first put directly into the amplifier of the polygraph, and the driver output of the pen, which "integrated" the signal (see figure 4), was then input into the computer at the occurrence of the R-wave of the ECG signal.

Conditioned Responses

During CER trials, only the tone (CS+ or CSr) was presented to the rat; no footshocks were delivered. A speaker was placed over the rat's home cage and

connected to the computer controlled stimulator which produced the tones (10 sec, 80 db, 950 or 300 Hz) during conditioning. After determining that the animal was at a "resting" or baseline status (via inspection of HR & BP), the trial was initiated. Data was recorded for 750 data points (approximately 2 min), with the tone CS beginning during second 21; seconds 9 to 19 served as the baseline for that trial. Responses were tested under three conditions, each constituting a different category:

A. CS+ and CSr alone (RSA data collection)

(All groups)

B. CS+ and CSr with baroreflex gain testing

(Groups 1 & 2)

C. CS+ and CSr with pharmacological blockade

(Groups 1 & 2)

The specific procedures and order of CER presentation for each of the above categories is fully explained in the appropriate chapters.

US Presentation

Following all CS+ and CSr trials, responses were recorded during the delivery of the footshock alone (US).

Sacrifice

At the completion of all necessary testing, the rat was anesthetized with an overdose of sodium pentobarbital (300 mg/kg) administered through the venous line. The rat was perfused transcardially with a 10% formalin solution, and the brain removed and allowed to sink in 30% sucrose in formalin solution. Frozen 50 um sections were cut and stained with cresyl violet for histological reconstruction of the lesion sites.

DATA ANALYSIS

The average response patterns for the four parameters (BP, HR, RESP, VOC) measured during the behavioral trials, the baroreflex gain values, and the pharmacological trials were calculated for each group and compared as described below. Responses were considered significantly different if the calculated p value was less than 0.05. All significant responses were reported as mean $\pm$ sem.

CS Responses

Statistical comparisons were made between the four experimental groups' mean arterial pressure (MAP), heart rate (HR), respiratory rate (RR), and ultrasonic vocalization (USV) during the behavioral trials under the first experimental condition outlined previously. Each parameter was averaged within each trial on a second by second basis over the 120 seconds of each animal's CS+

and CSr trial. To permit across animal averaging, responses in all four parameters were determined as changes from second 20. Baseline values were determined as the average value of the parameter studied in seconds 9 to 19. This yielded group average blood pressure, heart rate, respiration rate and ultrasonic vocalization frequency tracings with their corresponding standard errors (SEM) for the CS+ and CSr trials. Respirations were averaged on a 2 second basis to eliminate the possibility of 0 responses/sec. Comparisons within each group to define statistically significant responses (changes from baseline) was made by multiple comparisons (Bechhofer-Dunnet) between the mean baseline responses (sec 9-19) and each of the following 11 second bins: 21-31; 32-42; 43-53; 54-64; 65-75; 76-86; 87-97; 97-108 for each parameter studied. Comparisons between groups was made by ANOVA, followed by t-tests between corresponding time periods: baseline (sec 9-19), CS (sec 21-31), and late CER responses (sec 40-120).

Baroreflex Responses

The peak differences in BP and HR produced by balloon inflations were determined for each of the four time points studied (seconds 12, 27, 37 and 82) for CS+ and CSr responses. An individual gain was then calculated (dHR/dBP) for each animal at each time point. The median value of each time point was calculated for each group, resulting in the baroreflex gain at each of the four time points for Control and MFC lesioned groups. The raw signals were then averaged second by second over the entire 120 seconds for each trial. To permit across

animal averaging, responses were determined as changes from baseline (baseline defined as the mean value of seconds 3 to 10). This yielded group average BP and HR tracings with their corresponding standard errors for CS+ and CSr responses. Comparisons were made within each group at the four time points to define statistically significant responses (changes from time point 1) by multiple comparisons (Bechhofer-Dunnet). Comparisons between groups was made by ANOVA, followed by t-tests between corresponding time points. Comparisons between the computed gains was similarly performed.

Pharmacological Responses

For the third condition studied (CER with pharmacologic blockade), HR and BP were averaged second by second over the entire 120 seconds. Each sub-category (atropine, atenolol, or atropine + atenolol) was then pooled, forming three sub-groups. Within group comparisons (multiple comparisons) were performed to determine any statistically significant responses (changes from baseline) as previously described for CS responses. Between group comparisons were performed, comparing animals with CER + pharmacologic blockade (category C) with animals receiving only the CS (category A) using ANOVA and multiple comparisons in order to characterize the response.

Calculation of Variability

The variability of BP and HR parameters was calculated for baseline (sec 9-19), CS (sec 21-31) and late CER responses (sec 40 to 120) by determining the standard deviation of the parameter in question for each animal at each of the three periods studied. The mean variability was then calculated from these individual animal values, yielding a measure of variability $\pm$ sem for each period in each of the groups studied (Control, MFC, SAD and SAD + MFC).

Figure 3. Analysis of blood pressure signal sampling. Measurement of the blood pressure signal at the peak of the R-wave (solid arrows) in the heart rate signal corresponded to approximately 50% of the systolic/diastolic range (open arrows). Bar = 0.5 seconds.

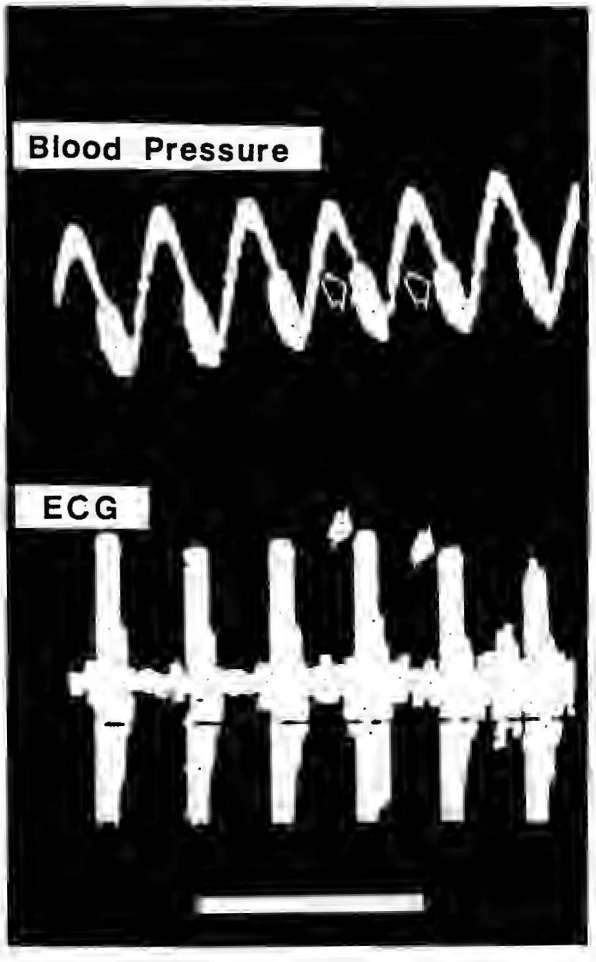
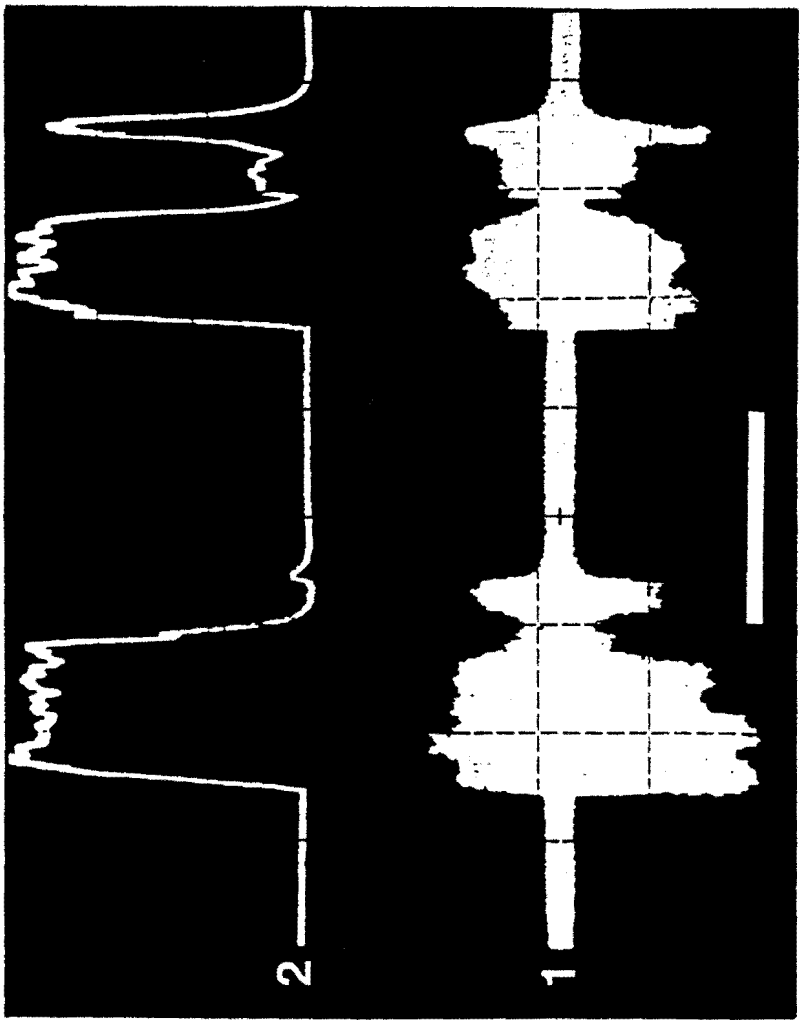

Blood Pressure
ECG

Figure 4. Integration of the microphone signal by the polygraph. Photograph of oscilloscope tracings displaying the raw microphone input signal (1) and the "integrated" signal produced by the driver output of the polygraph (2). Signal 2 was the signal received by the computer for storage. Bar = 0.4 seconds.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CHAPTER V

CARDIOVASCULAR CONDITIONED EMOTIONAL RESPONSES

INTRODUCTION

Lesions of the hypothalamus and amygdala, which project directly to brainstem cardiovascular "centers" such as the solitary nucleus and nucleus ambiguus, attenuate the blood pressure and heart rate responses of the conditioned emotional response (CER; Cohen, 1975; Kapp et al., 1979; Smith et al., 1980; Francis et al., 1980; Buchanan & Powell, 1982a; Pardini et al., 1986; Gentile et al., 1986). The infralimbic region of the medial frontal cortex also projects to brainstem autonomic regions such as the solitary nucleus (van der Kooy, 1982; Terreberry & Neafsey, 1983; 1987), but no studies of the effects of infralimbic lesions on the CER have been performed. Electrical or chemical stimulation of the MFC elicits prominent cardiovascular and other autonomic changes (Kaada, 1960; Lofving, 1961; Terreberry & Neafsey, 1984; Buchanan et al., 1984, 1985; Burns et al., 1985; Hurley-Gius & Neafsey, 1986; Hardy & Holmes, 1988). In addition, stimulation of the medial frontal cortex prior to electrically induced defense responses (via stimulation of the amygdala) results in attenuation or elimination of the defense response (Al Maskati & Zbrozyna, 1989). For these reasons, the present study investigated the effect of lesions of the infralimbic portion of the medial frontal cortex on the cardiovascular, respiratory and vocalization responses during the CER. In order to distinguish the learned (associative) components of the CER from the unlearned (nonassociative) components of the orienting response, the responses to a second tone (CSr) that was randomly paired with the footshock were also studied.

The specific aims of this portion of the study were to describe the CS+ and CSr cardiovascular conditioned emotional responses in the normal rat and to determine the sympathetic and parasympathetic contributions to these normal responses by selective pharmacological blockade with methyl atropine and atenolol. Comparisons were then made to MFC lesioned rats to determine whether these lesions altered the normal response pattern, and if so, through what sympathetic and/or parasympathetic contributions the response was mediated. Finally, to determine if the responses seen were influenced primarily by the learned (associative conditioning) or the unlearned (nonassociative conditioning) component of the cardiovascular conditioned emotional response, comparisons were made between the CS+ and CSr responses.

METHODS

The procedures used for conditioning, cannula implantation, data recording, animal sacrifice and data analysis have been previously discussed in the "General Methods" section (Chapter 4) of this dissertation.

MFC Aspiration Lesion Study

A pilot study involving large aspiration lesions of the MFC was performed to determine whether any change in the response pattern would be found. The medial frontal cortex was removed bilaterally from 2 to 5 mm anterior to bregma, 1 to 2 mm laterally from the midline, and 3 to 5 mm ventral from the surface in ketamine HCl (100 mg/kg) anesthetized rats. Controls consisted of sham-operated animals. The animals were allowed to recover for two weeks. Following recovery, the animals were then conditioned to a single CS+ tone (800 Hz, 10 second duration) followed immediately by a 2 milliamp scrambled footshock. The animals received 10 orientation trials (no footshock), followed by 30 acquisition trials (tone paired with footshock). The intertrial interval was approximately 2 minutes. Electrodes and cannulas for recording HR and BP were implanted immediately after conditioning. The following day, BP and HR were recorded during three CER trials. Following these trials, the animals were sacrificed by anesthetic overdose and cardiac perfusion, and the brains examined histologically.

MFC Chemical Lesion Study

Animals were anesthetized with ketamine HCl (100 mg/kg) and placed in a stereotaxic frame fitted with blunted earbars to prevent damage to the rat's eardrums. The skin over the MFC was incised and a topical anesthetic applied. A small burr hole was made in the skull bilaterally (anterior 3.5 mm from bregma, lateral 0.75 mm from midline). A micropipette attached to a Hamilton syringe was lowered 4.0 mm from the surface into the ventral medial frontal cortex. Lesions were created by bilateral microinfusions (0.4 μl over 10 min.) of the neurotoxin N-methyl-D-aspartate (100 nmol; Verberne et al., 1986). NMDA destroys neuronal cell bodies while sparing axonal fibers of passage (Schwarcz, 1978; Zaczek, 1982). Shams received sterile saline. The pipette was then removed, the holes in the skull filled with gel-foam and the wound sutured. The animal was returned to his own cage to recover for two weeks.

Conditioned Responses

Responses to the CS+ and CSr were tested under two conditions. During CER's in the first condition (category A), only blood pressure, heart rate, respiration, and vocalization responses were recorded during the two minute analysis period. This period included a 20 second pre-CS (baseline) epoch, a 10 second CS presentation epoch, and a 90 second post-CS epoch.

During the CER's in the second condition (category B), the response was analyzed following the administration of specific pharmacologic blocking agents.

Conditions for CER presentation were identical to those in category A. Control and MFC lesioned rats were randomly divided into three sub-categories (N = 4 for each). CER's were tested 5 minutes after the administration of the following drug combinations: (a) the muscarinic cholinergic blocker methyl atropine (1 mg/kg); (b) the cardio-selective beta 1 antagonist atenolol (1 mg/kg); (c) methyl atropine + atenolol (1 mg/kg each). The efficacy of methyl atropine blockade was determined by measuring the bradycardia elicited by a rise in blood pressure caused by inflation of the aortic balloon catheter. The efficacy of atenolol blockade was determined by measuring the tachycardia elicited by administration of a bolus (1 μg/kg) of isoproterenol, a beta adrenergic agonist which increases HR, cardiac output, coronary and peripheral vascular smooth muscle vasodilation via sympathetic activation.

Chronology of Procedures:

1. MFC lesion/sham - 2 week recovery period
2. Classical conditioning of animal, followed by implantation of cardiovascular and respiratory recording hardware - 24 hour recovery
3. Recording of heart rate, blood pressure, respiratory rate and ultrasonic vocalization during the CER
4. Recording of responses to the footshock (US) alone
5. Sacrifice by anesthetic overdose
6. Data analysis

MFC Aspiration Lesion Responses

Histological reconstruction of the lesions yielded three distinct groups:

1. Controls (N = 11)
2. Dorsal lesions (N = 5)
3. Dorsal + Ventral lesions (N = 6)

Figure 5A illustrates coronal sections through dorsal + ventral (1) and dorsal-only (2) MFC lesioned animals. Note that the infralimbic portion of the MFC (immediately dorsal to the taenia tecta - tt) was intact in the dorsal group, but was totally eliminated in the dorsal + ventral group. Figure 5B represents the total rostral-caudal and ventral extents of the lesions from a sagittal view.

Average responses of the first CER trial for all three groups are shown in figure 6 and table 3. Controls responded to the CS alone with a pressor response paired with a tachycardia. The dorsal MFC lesioned group responded to the CS with a similar increase in BP coupled with an increased tachycardia, which was significantly different from baseline immediately following the offset of the CS and throughout the remainder of the trial. The dorsal + ventral MFC lesioned group responded to the CS with a pressor response similar to Controls and a clear bradycardia. The bradycardia was significantly different from baseline, Controls, and Dorsal MFC lesioned rats throughout the remainder of the trial (40 sec).

Lesion Analysis

Figure 7 is a photomicrograph of a coronal section through the lesion site of animal QM10 at the level of the infralimbic cortex illustrating that the neuronal cell bodies of the infralimbic cortex were destroyed, but the general morphology of the cortex was not disturbed. Figure 8 represents left and right sagittal views of the extent of the individual lesions for all of the animals in this group (N = 11). In all cases, the infralimbic portion of the MFC was eliminated. Figure 9 illustrates the areas common to all lesions (solid line) and the largest area lesioned (dotted line). These lesions did not affect the resting BP or HR values as compared to resting Control values (see Table 4).

CER Responses

Polygraph tracings of the cardiovascular, respiratory and USV responses during single CS+ or CSr trials are illustrated in figure 10. The response during the CS+ for Control rats was a pressor response linked with a tachycardia (A). Following the tone, there were obvious, long lasting changes in respiration and USV. The MFC lesioned response to the CS+ (C) was a pressor response and, in this case, a bradycardia. Note that there were no ultrasonic vocalizations, and the respiratory pattern was not greatly altered following the CS+. The increased beat-to-beat HR variability seen in Controls following the CS+ is also conspicuously lacking in MFC lesioned rats. The cardiovascular responses to the CSr presentation were generally smaller than those during the CS+ (Figure 6B and D). At the onset

of the CSr, both BP and HR responses appear smaller than those during the CS+. Following the CSr offset, BP and HR show considerably less beat-to-beat variability than CS+ responses. Note also the absence of ultrasonic vocalizations and respiratory changes in both groups following the CSr.

Average Responses

The blood pressure responses to the CS+ for both groups were nearly identical (Figure 11A and Table 5), consisting in a pressor response which was significantly greater than baseline. The average heart rate response to the CS+ (Figure 11B) for Controls was a tachycardia, but for MFC lesioned rats it was bimodal, consisting of a small initial increase in HR followed by a bradycardia beginning at second 25. Due to this bimodality, the MFC CS+ HR response was not significantly different from baseline (Table 5), but was significantly less than CS+ Controls. The mean BP and HR responses during the late phase (seconds 40 to 110) of the CS+ were not significantly different from baseline.

Variability, measured by calculating the mean of each animal's standard deviation during each period, is shown in table 6. Heart rate variability increased significantly in the late phase compared to baseline following the CS+ in Controls, but was not significantly different from baseline for MFC lesioned rats.

The average CSr blood pressure and heart rate responses are shown in figure 11C and D. While the bimodal pattern of the BP response is similar to that of the CS+ response, the mean pressor response during the CSr was significantly

smaller than the CS+ response for both groups (Table 5). The average CSr heart rate response did not differ from baseline at any time throughout the trial for either group, and variability was not significantly different from baseline during the late CSr phase (Table 6).

CS+ Pharmacological Blockade

Methyl atropine significantly raised the mean resting HR ($p<0.01$) compared to untreated animals for both groups (Table 4); mean resting blood pressure was not affected. The average CS+ blood pressure responses under methyl atropine in Control and MFC lesioned rats did not differ (Figure 12A). The magnitude of the average heart rate response in MFC lesioned rats was significantly reduced compared to Controls (Figure 12B). The mean atropine-treated MFC CS+ heart rate response during the CS+ was 8.1 $\pm$ 1.8 bpm, while Controls responded with a 17.2 $\pm$ 3.8 bpm increase; this represents a 52% decrease. Controls remained significantly above baseline throughout the trial.

Administration of atenolol did not significantly affect resting blood pressure or heart rate (Table 4). During the CS+ under atenolol, the BP response in Controls appeared to be greatly increased compared to MFC lesioned rats, due to the unusually large response by one animal (Figure 12C). The responses, however, were not significantly different between the two groups. The heart rate response (Figure 12D) under atenolol for both groups was a small, initial decrease (arrow), followed by a slow-onset bradycardia, with the peak decrease occurring near the

offset of the CS+. The initial decrease was most likely due to baroreceptor activation, while the more slowly developing bradycardia was due to direct parasympathetic activation. Heart rate returned to baseline by second 50.

The combined effects of methyl atropine and atenolol did not significantly affect resting BP or HR levels (Table 4); variability in the resting signals was almost completely eliminated (Table 6). The blood pressure CS+ responses for both groups under atropine + atenolol were not significantly different from one another (Figure 13A). The mean heart rate responses for both groups were not significantly different from baseline, although a small increase was noted. Neither BP or HR was significantly different from baseline throughout the remainder of the trial following the CS+ (Figure 13B).

CSr Pharmacological Blockade

Pharmacological analysis of the CSr BP and HR responses are shown in figure 14. The average CSr blood pressure patterns in Control and MFC lesioned rats (Figure 14A) following administration of methyl atropine were not significantly different from baseline or each other. The MFC CSr HR response was significantly reduced compared to atropine-treated CSr Controls, corresponding to a 73% reduction in sympathetic tone during the CSr for MFC lesioned animals (Figure 14B). Under atenolol, BP and HR responses were not significantly different from one another or baseline during the CSr. Note, however, that heart rate CSr responses (Figure 14D) were significantly different from CS+ HR responses under

atenolol (Figure 12D), indicating a lack of parasympathetic activation during the CSr.

Unconditioned Stimulus (US) Responses

Following all trials, the rats were placed back into the plexiglass shock chamber where acquisition occurred. The animals each received one US (2 mAmp footshock, 0.5 sec duration). In all cases, the US response consisted of a large increase in blood pressure paired with a strong tachycardia. Ultrasonic vocalizations began immediately following the US and persisted vigorously through the end of the trial, lasting up to 10 minutes in some cases. The patterns of these responses in rats with MFC lesions were not different from those seen in Controls or SAD lesioned rats. The tachycardia and occurrence of USV during the US trial was significantly different, however, for the MFC lesioned group when compared to the MFC CS+ response, which consisted of a slow-onset bradycardia and no USV.

DISCUSSION

Conditioned Responses

Cannon (1927) postulated that when an organism faces a threatening situation, bodily resources are mobilized to prepare the animal for "fight or flight." In these situations, the sympathetic nervous system is activated. It is well established that aversive emotional arousal and stress are often accompanied by widespread sympathetic activation in experimental animals and man. Using the conditioned emotional response paradigm, Smith et al. (1979) reported that propranolol blocked the CS-evoked tachycardia in baboons, while the conditioned tachycardia in dogs was almost completely abolished by beta-1 adrenergic blockade (Billman & Randall, 1981). Obrist et al. (1972) attributed sympathetically-mediated heart rate increases to the expectation of stress. This is further supported by recent evidence which directly recorded increases in neural activity from single sympathetic cardiac postganglionic neurons during conditioning (Cohen, 1982).

Conditioned increases in sympathetic activity are often accompanied by a synergistic decrease in vagal inhibition of the heart (Cohen & Randall, 1984). Dykman and Gantt (1959) found that the classically conditioned heart rate acceleration in dogs was differentially controlled by the autonomic nervous system; the parasympathetic component was responsible for the short latency increase in heart rate, while the sympathetic component was responsible for a longer latency acceleration. Comprehensive studies in both the pigeon (Cohen & Pitts, 1968) and rat (Iwata & LeDoux, 1988) concluded that the conditioned heart rate acceleration

was due to coactivation of the sympathetic and parasympathetic nervous systems. Direct recording of vagal cardiac postganglionic neurons in the pigeon during conditioned tachycardia revealed an initial decrease in discharge at the onset of the CS, followed by a return to baseline levels near the end of the CS presentation (Gold & Cohen, 1981). Short latency heart rate changes are generally mediated by alterations in vagal tone, while longer latency responses, i.e., conditioned responses, are mediated by both sympathetic and parasympathetic activity on the same trial. Those stressors which require controlling responding to avoid aversive or noxious stimuli have been shown to have the most pronounced effects on the cardiovascular system (Galosy et al., 1983), during which sympathetic influences may totally override vagal influences (Grossman, 1983). The responses presented here agree with these findings and support all previous reports of the cardiovascular responses during the CS+ in freely moving rats (McDonald et al., 1963; Fehr & Stern, 1965; Black & Black, 1967; Duncan, 1972; LeDoux et al., 1984, 1986; Iwata & LeDoux, 1988), namely a pressor response paired with a tachycardia. The major contribution to the aversively conditioned tachycardia in Controls was increased sympathetic outflow buffered by a slow-onset activation of the parasympathetic component during the CS+. This long-latency, autonomic response far outlasts the actual CS+ presentation. The initial decrease in heart rate at the onset of the tone was most likely due to baroreceptor activation; the long latency activation of the parasympathetic component acts to buffer the increases in sympathetic outflow during the late portion of the CS+ trial.

Lesions of various limbic structures within the CNS result in significant changes in the autonomic and/or behavioral conditioned responses. Sympathetically mediated cardiovascular responses are controlled by the lateral hypothalamus (LeDoux, 1987), and both electrolytic and chemical lesions of this area are known to significantly decrease the conditioned arterial pressure response (Iwata et al., 1986; LeDoux et al., 1988). Behavioral responses, such as freezing, were not affected by hypothalamic lesions, but lesions of the central gray significantly reduced the freezing behavior in rats. Lesions of the amygdala, which is known to project to both of these structures as well as the MFC, resulted in attenuation of both the autonomic (cardiovascular) and behavioral (freezing) conditioned responses (Gentile, 1986; Vanderwolf et al., 1988; Galeno et al., 1984; LeDoux et al., 1988). Lesions of other CNS structures and their effects on conditioned responses were summarized previously in Table 1.

Lesions of the MFC resulted in various heart rate responses, dependent on the extent and placement of the lesion. Figure 15 displays BP and HR responses during the CS+ for all groups studied. Aspiration lesions of only the dorsal MFC (prelimbic and anterior cingulate areas) indicated that the dorsal MFC may normally act to **decrease** HR during stress. Al Maskati & Zbrozyna (1989) reported electrical and chemical stimulation sites in the dorsal MFC, corresponding to the prelimbic regions, which they classified as "sympatho-inhibitory" areas. Stimulation of these areas resulted in attenuation or inhibition of the defense response and the associated cardiovascular responses. Aspiration lesions of the dorsal + ventral

(infralimbic) MFC indicated that the ventral MFC may normally act to **increase** HR during stress. Chemical lesions of primarily the infralimbic portion of the MFC (with variable destruction of the prelimbic region) resulted in a bimodal HR response. In MFC lesioned animals, the early CS+ tachycardia under methyl atropine was reduced by 52% compared to atropine-treated Controls. This significant decrease in the amount of sympathetic activation during the CS+ in MFC lesioned rats implies that the ventral MFC is normally involved in activation of a significant number of sympathetic neurons during stress. Lesioning of the MFC eliminates this activation during the CS+, thereby reducing the outflow of the sympathetic system to the heart and significantly altering the HR response during the CS+. The rat MFC has been shown to project directly to the thoracic intermediolateral cell column (Hurley-Gius et al., 1986), the site of origin for preganglionic cardiac sympathetic neurons. It is possible, therefore, that the MFC influences sympathetic outflow directly. Through its projections to the solitary nucleus and lateral hypothalamus, the MFC also has indirect access to the sympathetic nervous system.

Figure 16 illustrates a possible neuroanatomical explanation for our results. At rest, the ventral MFC tonically modulates output in the baroreflex arc at the level of the NTS, affecting the gain of the reflex so that increases in BP are buffered by corresponding decreases in HR; the system is, in a sense, "balanced." Controls respond to stress via activation of the MFC and other cardiovascular centers, such as the hypothalamus and amygdala, resulting in a large sympathetic outflow. The

"sympatho-inhibitory" effects of the dorsal MFC are not sufficient to overcome the increases in sympathetic outflow achieved by the activation of the ventral MFC, hypothalamus and amygdala. The short latency parasympathetic activation may be inhibited (parasympathetic "withdrawl")(Cohen & Randall, 1984), while long latency, direct activation of parasympathetic neurons by central cardiovascular centers is unaffected. The sympathetic outflow is dominant, and the resulting response is a tachycardia buffered by parasympathetic outflow.

In MFC lesioned animals, the direct and indirect activation of the sympathetic component by the ventral MFC is lost. Due to inhibition (possibly by the dorsal MFC) of the direct pathways to the parasympathetic system and the reduced gain of the baroreceptor reflex due to loss of the ventral MFC, a temporary reduction in parasympathetic outflow occurs. Coupled with direct activation of the sympathetic system via the amygdala and hypothalamus, a small HR increase results at the onset of the CS+. As activation (both direct and indirect) of the parasympathetic system occurs, the parasympathetic system becomes dominant, and a slow onset bradycardia is observed late in the CS+, and persists after termination of the CS+ tone.

CSr Responses

Our CS+ and CSr BP responses agree with data presented by Iwata and LeDoux (1988), who described significant attenuation of the BP response with all forms of nonassociative conditioning (random, backwards, US-alone, naive)

compared to associative (paired) conditioning. Our CS+ and CSr HR responses, however, differ from Iwata and LeDoux's finding that the HR response to nonassociative conditioning (CSr) was not significantly altered compared to associative conditioning (CS+). They concluded that the BP response clearly and reliably reflects the associative conditioning process in rats, whereas the HR response reflects a nonassociative process. Our results show a significant reduction in the HR response to random (nonassociative) conditioning compared to that of paired (associative) conditioning, in agreement with several other studies that have reported HR responses which differ significantly between associative and nonassociative trials (Fitzgerald & Martin, 1971; Buchanan & Powell, 1982 a & b; Martin & Fitzgerald, 1980). This disparity may be explained by noting that, in determining both associative and nonassociative responses, Iwata and LeDoux averaged the effects of three trials, a procedure that increases the significance of the second and third trials during which extinction is occurring. Our study utilized only the initial trial, and therefore the effects of extinction were not averaged into the results.

Responses in MFC lesioned animals during the CSr were significantly different than the CS+ responses. The CSr response, mediated only by the sympathetic component, resulted in a tachycardia in MFC lesioned rats. Without a parasympathetic component, MFC lesioned rats could not display a bradycardia during or following the CSr. These results are consistent with those of Iwata & LeDoux (1988), who determined that the CS+ response was due to coactivation of

the sympathetic and parasympathetic systems, whereas the CSr response was due only to sympathetic activation.

US Responses

The responses associated with the US indicate that the "learned" response to the CS+ and CSr were mediated by brain areas other than or in addition to the brain areas mediating the US response. During the US, it is likely that pain pathways and other sensory input contributed to the profound pressor response, tachycardia and vocalizations.

CONCLUSIONS

The normal CS+ response was a pressor response paired with a tachycardia mediated by coactivation of the sympathetic and parasympathetic nervous systems, in which the sympathetic component was dominant, resulting in the observed tachycardia. The normal CSr response was a reduced pressor response paired with a significantly reduced tachycardia (compared to CS+ Controls) mediated only by the sympathetic nervous system. The sympathetic component seen in the CSr response was significantly smaller than that of the Control CS+ response.

The CS+ response in MFC-lesioned animals was dependent on the extent and type of lesion incurred. Blood pressure responses were not significantly different from Control CS+ responses for any of the groups studied. **Dorsal** aspiration lesions resulted in an increased HR response (compared to CS+ Controls); **Dorsal + Ventral** aspiration lesions resulted in a bradycardia, which was significantly different from CS+ Controls; **Chemical Ventral** lesions resulted in a small HR increase followed by a slow-onset bradycardia. During the CER, sympathetic outflow was significantly reduced in ventral lesioned animals; parasympathetic outflow was not noticeably affected. The ventral MFC is thought to control sympathetically-mediated HR increases during stress, influencing the learned (associative) component of the CER; the dorsal MFC is thought to mediate sympatho-inhibitory influences on HR responses during stress.

Figure 5. MFC aspiration lesions. A. Coronal sections taken every 1 mm anterior to bregma representing typical lesions of (1) Dorsal + Ventral MFC lesion and (2) Dorsal MFC lesion. Bar represents 1 mm. Note sparing of infralimbic cortex (*) in (2). B. Sagittal view of the rat brain showing rostral-caudal and ventral boundaries of the largest lesion in each group. ac-anterior commissure; F-fornix; cc-corpus callosum; tt-taenia tecta; b-bregma

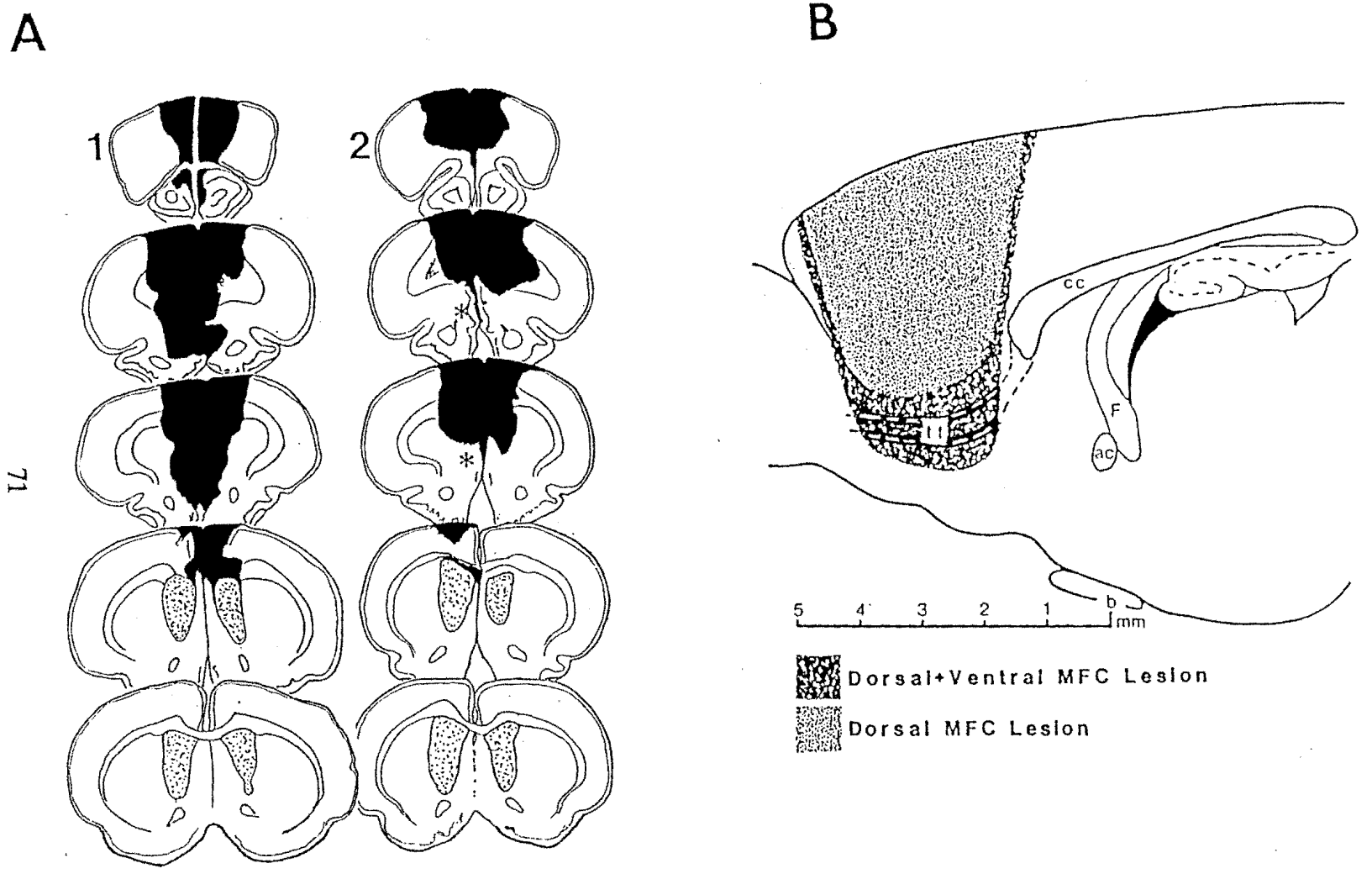
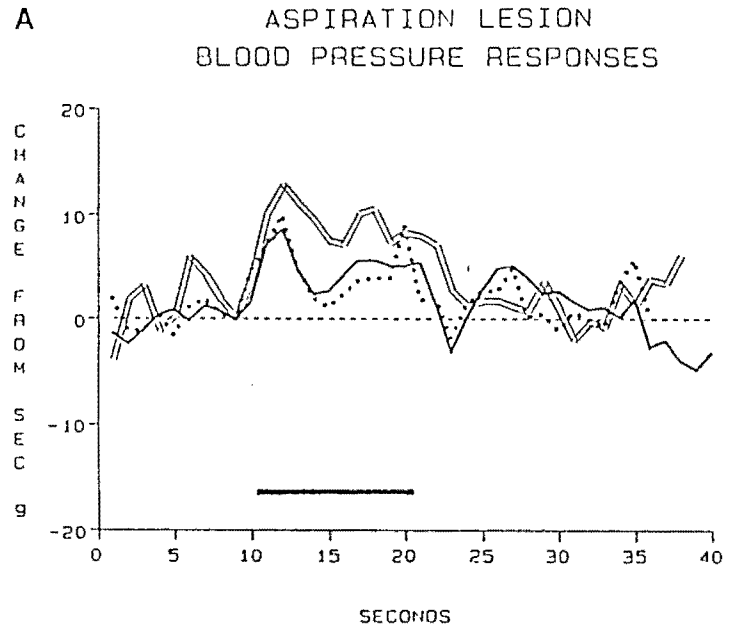

A
1
2
B
cc
F
ac
5
4
3
2
1
b
mm
Dorsal+Ventral MFC Lesion
Dorsal MFC Lesion

Figure 6. Average blood pressure and heart rate responses to CS following aspiration lesions of the MFC. All responses are plotted as change from baseline over time (seconds), with baseline computed from seconds 5 to 10. Solid lines represent Control responses; dotted lines represent Dorsal MFC lesions; double lines represent Dorsal + Ventral MFC lesions. A. Blood pressure responses. B. Heart rate responses.


A
ASPIRATION LESION
BLOOD PRESSURE RESPONSES
CHANGE FROM SEC g
20
10
0
-10
-20
0
5
10
15
20
25
30
35
40
SECONDS

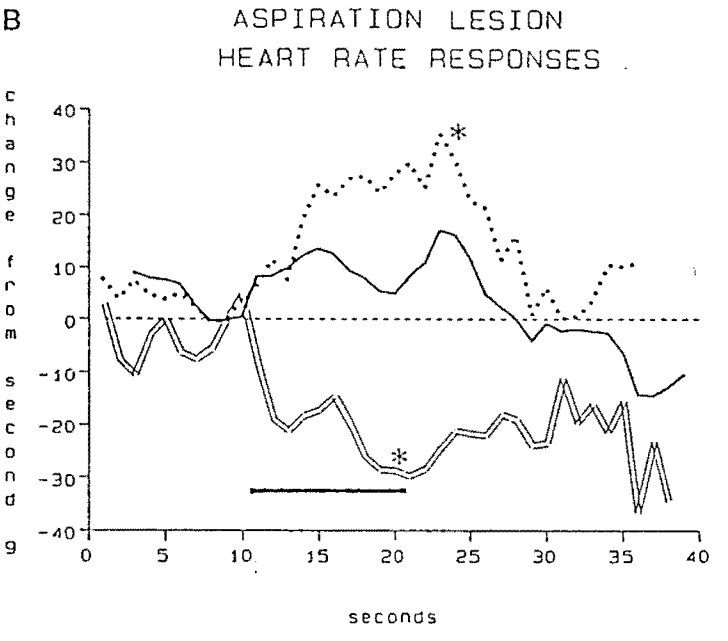

B
ASPIRATION LESION
HEART RATE RESPONSES
change from second g
40
30
20
10
0
-10
-20
-30
-40
0
5
10
15
20
25
30
35
40
seconds

Table 3. **MEAN CARDIOVASCULAR CS RESPONSES FOLLOWING ASPIRATION LESIONS**

		BLOOD PRESSURE RESPONSES		HEART RATE RESPONSES	
GROUP	N	BASELINE	CS	BASELINE	CS
Control	11	-0.7 ± 0.8	7.6 ± 1.9*	0.3 ± 3.9	13.1 ± 3.4*
D+V MFC	6	2.0 ± 3.4	9.3 ± 3.6*	-5.0 ± 3.8	-21.1 ± 8.5*
D MFC	5	0.2 ± 0.8	4.7 ± 2.7	3.5 ± 2.7	21.2 ±12.1

Mean blood pressure and heart rate values ± 1 standard error of the mean (SEM). D+V MFC denotes dorsal + ventral MFC aspiration lesion; D MFC denotes dorsal-only MFC lesion. Baseline values computed from seconds 1 to 10; CS values computed from seconds 11 to 20; Responses significantly different from baseline values ($p<0.05$) indicated by *. For other significant comparisons, see text.

Figure 7. Photomicrograph of the rat medial frontal cortex taken 2.5 mm anterior to bregma following NMDA lesion in subject QM10. Note the lack of cells within the infralimbic cortex (above the taenia tecta), but that the integrity of the overlying prelimbic and anterior cingulate cortices has not been disturbed. IL, infralimbic cortex; PL, prelimbic cortex; AC, anterior cingulate cortex; tt, taenia tecta.

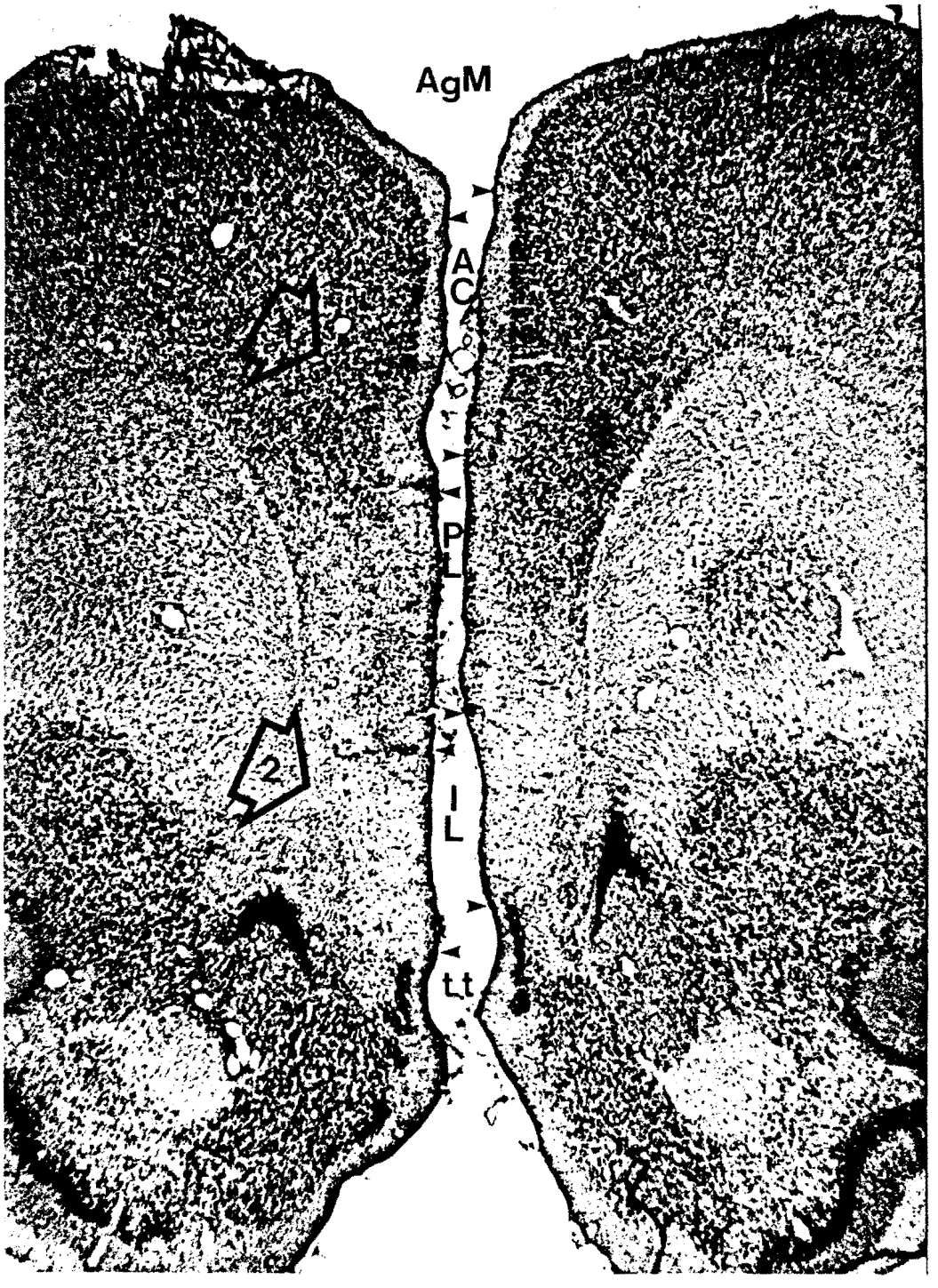

AgM
AC
PL
IL
tt
1
2

Figure 8. Sagittal views of left & right rat medial frontal cortex representing the extent of NMDA (chemical) lesions for each individual animal. In all cases, the infralimbic cortex (IL) was successfully ablated. A through K represent individual animal reconstructions of the lesions; L illustrates the cytoarchitectural boundaries used in this study. AC-anterior cingulate cortex; AgM-agranular medial cortex; CC-corpus callosum; IL-infralimbic cortex; MO-medial orbital cortex; PL-prelimbic cortex; a-anterior commissure; f-fornix; tt-taenia tecta.

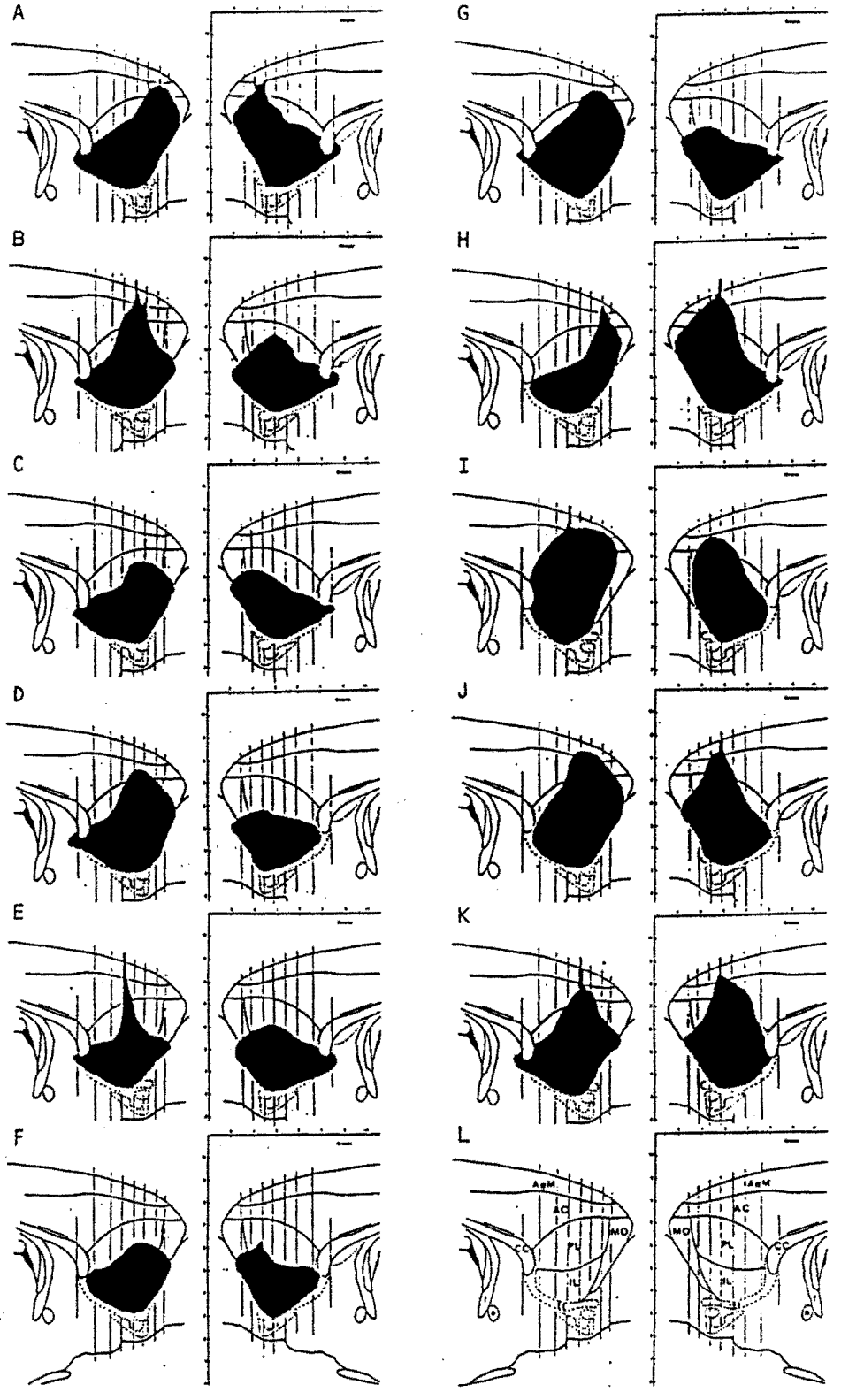

A
B
C
D
E
F
G
H
I
J
K
L
AgM
AC
MO
PL
IL
CC

Figure 9. Coronal view of left & right rat medial frontal cortex representing the extent of the area common to all lesions (hatched), and the largest composite lesion (dotted). AC-anterior cingulate cortex; AgM-agranular medial cortex; AgL-agranular lateral cortex; CC-corpus callosum; IL-infralimbic cortex; Ins-insular cortex; PL-prelimbic cortex; acb-accumbens; tt-taenia tecta.

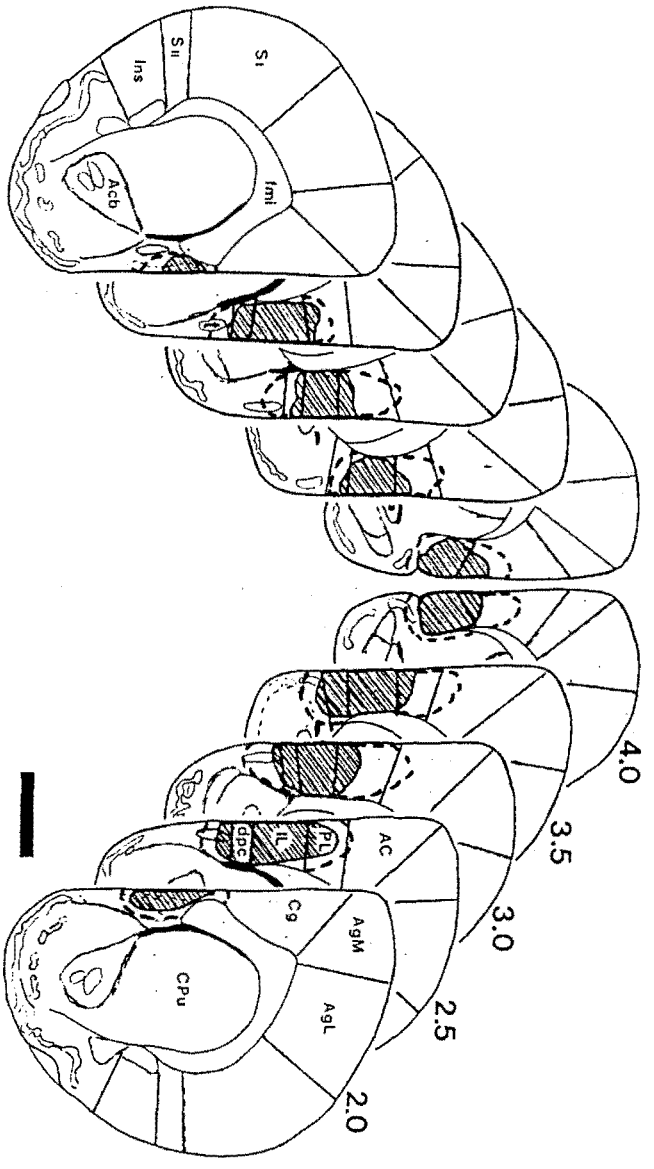

SII
SI
Ins
fmi
Acb
CPu
Cg
AgM
AgL
AC
GPe
4.0
3.5
3.0
2.5
2.0

Table 4.

RESTING BLOOD PRESSURE AND HEART RATE VALUES

GROUP (N)	BLOOD PRESSURE	HEART RATE
UNTREATED		
Control (13)	107.2 ± 2.5	364.4 ± 5.0
MFC (11)	108.2 ± 3.1	359.4 ± 4.9
METHYL ATROPINE		
Control (5)	109.4 ± 11.7	423.1 ± 17.4*
MFC (4)	110.1 ± 12.9	437.6 ± 22.7*
ATENOLOL		
Control (4)	105.1 ± 3.2	361.2 ± 5.8
MFC (4)	104.7 ± 3.8	357.3 ± 5.1
ATROPINE + ATENOLOL		
Control (4)	105.6 ± 1.7	362.3 ± 2.8
MFC (4)	106.1 ± 1.6	361.7 ± 2.0

Resting blood pressure and heart rate values ± 1 SEM before (UNTREATED) and after selective pharmacologic blockade. * denotes responses significantly different ($p<0.05$) from corresponding untreated group.

Figure 10. Polygraph tracings of individual Control and MFC lesioned CS+ and CSr responses. Blood pressure (BP), heart rate (HR), ultrasonic vocalizations (USV) and respiration (RESP) tracings; bar represents occurrence of 10 sec tone. A. Control responses during the CS+ presentation. B. Control responses during the CSr presentation. C. MFC lesioned responses during the CS+ presentation. D. MFC lesioned responses during the CSr presentation (oscillations appearing late in respiration (*) were due to movement artifacts).

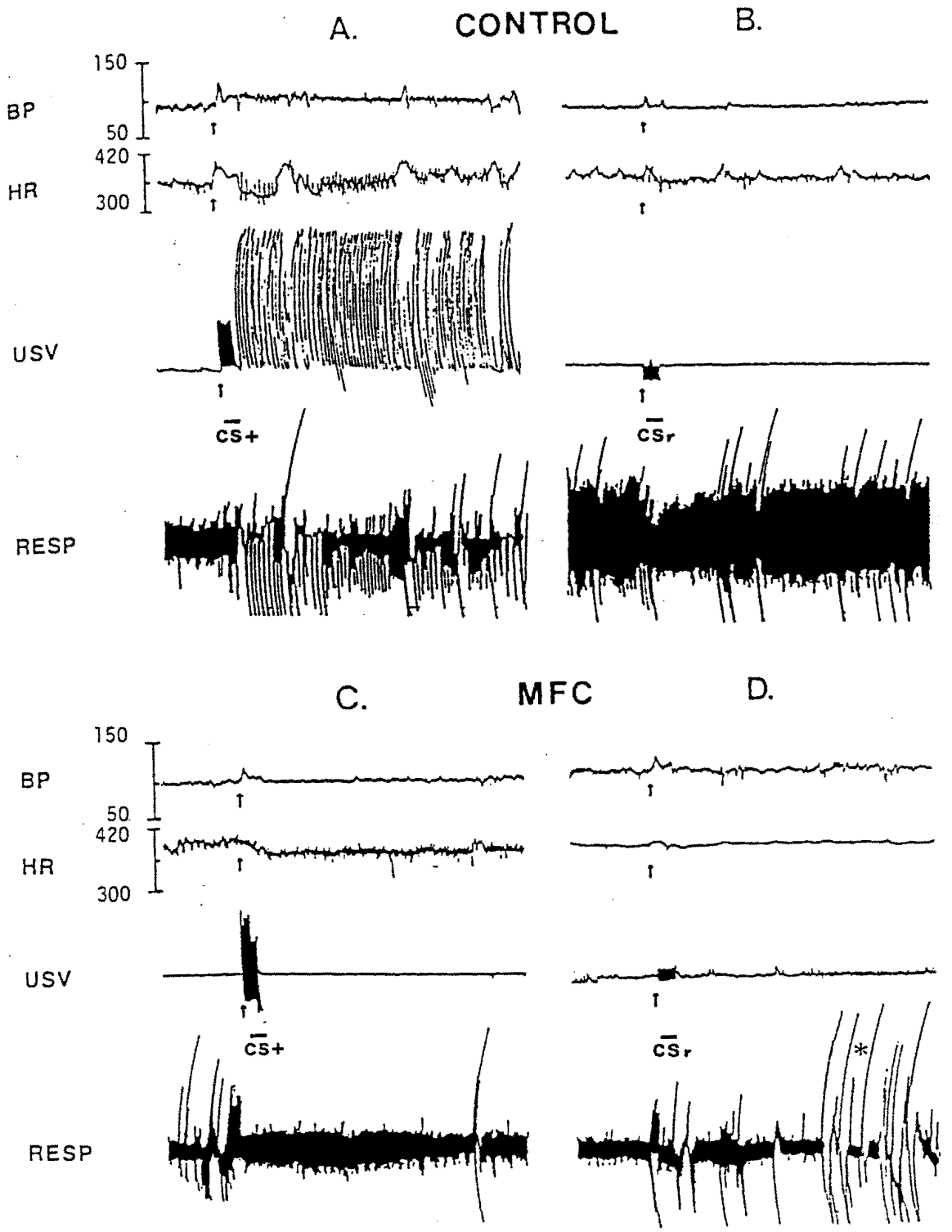

A.
CONTROL
B.
150
50
BP
420
300
HR
USV
CS+
CS-
RESP
C.
MFC
D.
150
50
BP
420
300
HR
USV
CS+
CS-
RESP
*

Figure 11. Average blood pressure and heart rate responses to the CS+ and CSr. All responses plotted as change from second 20 over time, with baseline computed from seconds 9 to 19. Dotted lines represent MFC lesions, solid lines represent Controls. Bars represent time of CS occurrence. * indicates MFC response significantly different ($p<0.05$) from Control. A. Mean CS+ blood pressure responses. B. Mean CS+ heart rate responses. C. Mean CSr blood pressure responses. D. Mean CSr heart rate responses.

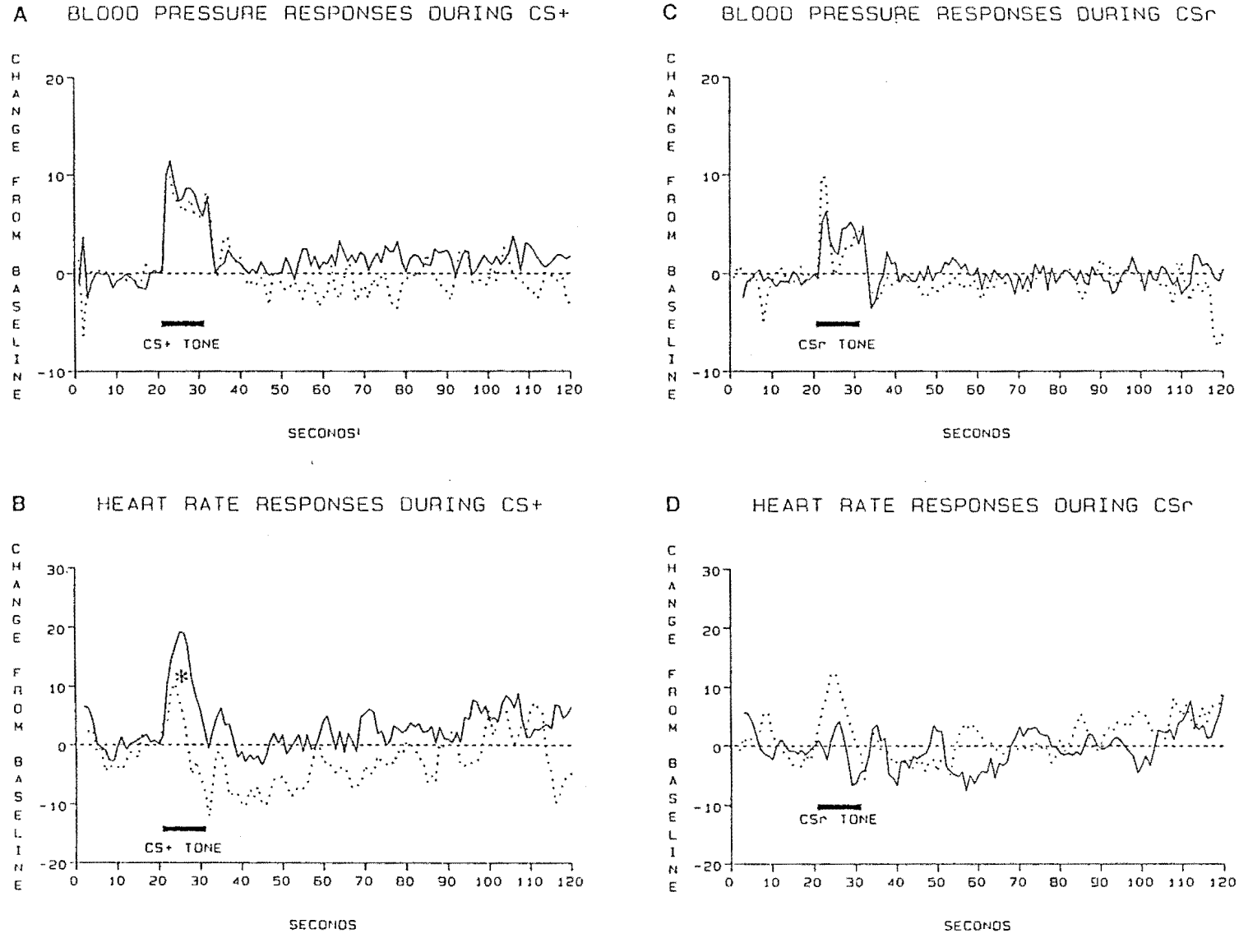

A BLOOD PRESSURE RESPONSES DURING CS+
CHANGE FROM BASELINE
20
10
0
-10
CS+ TONE
0 10 20 30 40 50 60 70 80 90 100 110 120
SECONDS
C BLOOD PRESSURE RESPONSES DURING CS-
CHANGE FROM BASELINE
20
10
0
-10
CS- TONE
0 10 20 30 40 50 60 70 80 90 100 110 120
SECONDS
B HEART RATE RESPONSES DURING CS+
CHANGE FROM BASELINE
30
20
10
0
-10
-20
*
CS+ TONE
0 10 20 30 40 50 60 70 80 90 100 110 120
SECONDS
D HEART RATE RESPONSES DURING CS-
CHANGE FROM BASELINE
30
20
10
0
-10
-20
CS- TONE
0 10 20 30 40 50 60 70 80 90 100 110 120
SECONDS

Table 5. Mean blood pressure and heart rate values $\pm$ 1 standard error of the mean (SEM). Baseline values computed from individual animal means of seconds 9 to 19; CS values computed from seconds 21 to 31. Responses significantly different from baseline values ($p<0.05$) indicated by *; responses significantly different between groups indicated by #.

Table 5. MEAN CARDIOVASCULAR CS+ RESPONSES

	BLOOD PRESSURE RESPONSES		HEART RATE RESPONSES	
GROUP	BASELINE	CS	BASELINE	CS
UNTREATED				
Control	-0.7 ± 0.8	7.6 ± 1.9*	0.3 ± 3.9	13.1 ± 3.4*
MFC	-0.3 ± 0.7	6.7 ± 1.7*	-1.6 ± 2.7	1.7 ± 4.4#
ATROPINE RESPONSES				
Control	-0.4 ± 0.5	2.5 ± 1.7	1.1 ± 2.1	17.2 ± 3.8*
MFC	-1.6 ± 1.5	3.7 ± 1.2*	3.8 ± 1.4	8.1 ± 1.8*#
ATENOLOL RESPONSES				
Control	1.3 ± 0.6	9.9 ± 7.5	-0.3 ± 1.8	-7.8 ± 3.7*
MFC	-0.3 ± 0.6	2.2 ± 1.2	-0.5 ± 2.4	-7.0 ± 2.4*
ATROPINE + ATENOLOL RESPONSES				
Control	0.3 ± 0.2	5.1 ± 2.4	0.5 ± 0.3	1.3 ± 0.7
MFC	-0.8 + 1.8	2.4 + 2.5	0.2 + 0.4	3.2 + 1.7

MEAN CARDIOVASCULAR CSr RESPONSES

UNTREATED				
Control	-0.6 ± 0.5	3.9 ± 1.7*	1.1 ± 2.4	2.5 ± 2.2
MFC	-0.4 ± 0.9	3.5 ± 1.9	-0.8 ± 2.7	6.1 ± 3.8
ATROPINE RESPONSES				
Control	-1.0 ± 0.6	1.5 ± 0.9	1.7 ± 1.6	14.3 ± 3.3*
MFC	1.0 ± 1.0	1.5 ± 1.4	0.6 ± 0.7	3.9 ± 1.5*#
ATENOLOL RESPONSES				
Control	1.7 ± 1.6	2.7 ± 2.9	-0.6 ± 0.6	-1.9 ± 2.1
MFC	0.1 ± 0.5	1.9 ± 1.3	-1.2 ± 2.2	-1.0 ± 3.7

Table 6. **CARDIOVASCULAR VARIABILITY MEASUREMENTS**

CS+ RESPONSES

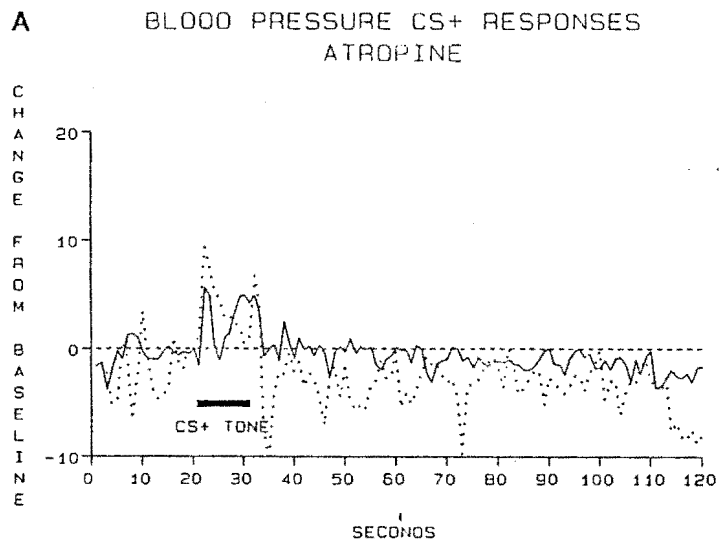
	BLOOD PRESSURE RESPONSES			HEART RATE RESPONSES		
GROUP	9-19	21-31	40-120	0-19	21-31	40-120
Control	2.4 ± 0.5	5.4 ± 0.9*	3.3 ± 0.5	4.1 ± 1.1	10.0 ± 1.2*	9.9 ± 1.3*
MFC	2.0 ± 0.5	4.7 ± 0.5*	4.3 ± 0.9	5.2 ± 1.3	8.3 ± 1.6	7.6 ± 1.2

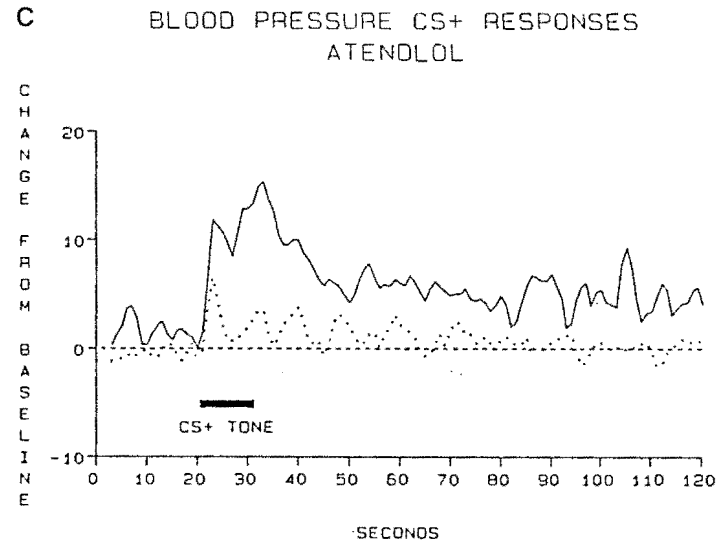
CSr RESPONSES

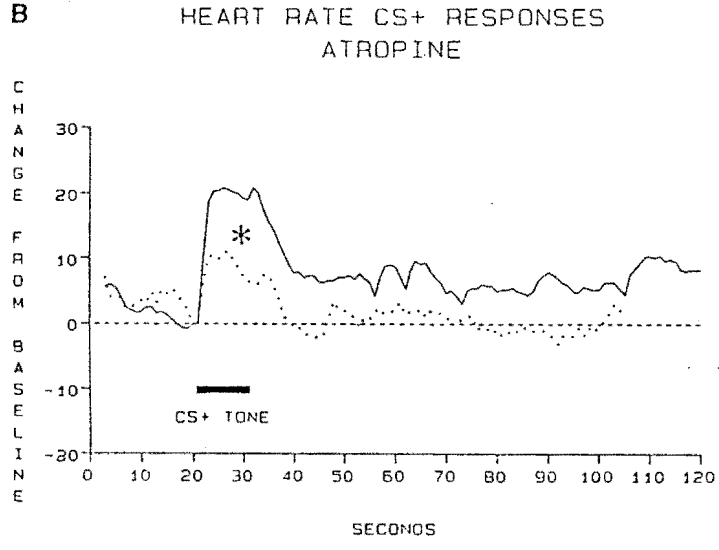
Control	1.5 ± 0.2	3.0 ± 0.5*	2.8 ± 0.6	5.1 ± 1.0	7.1 ± 1.3	7.5 ± 1.6
MFC	2.5 ± 0.5	5.4 ± 0.5*	2.9 ± 0.4	4.9 ± 1.5	7.1 ± 1.2	5.4 ± 0.7

Cardiovascular CS+ and CSr variability measurements expressed in mean standard deviation ± 1 SEM. Seconds 9-19 denote baseline period; seconds 21-31 denote CS period; seconds 40-120 denote late period. * denote responses significantly different from corresponding baseline.

Figure 12. Average blood pressure and heart rate responses to the CS+ during either atropine or atenolol treatment. All responses plotted as change from baseline over time, with baseline computed from seconds 15 to 20. Dotted lines represent MFC lesions, solid lines represent Controls. Bars represent time of CS+ tone occurrence. A. Mean blood pressure responses under methyl atropine administration. B. Mean heart rate responses under methyl atropine administration. * denotes MFC response was significantly less than Control. C. Mean blood pressure responses under atenolol administration. D. Mean heart rate responses under atenolol administration. Note that in both groups there is an inflection (arrow) in the bradycardic response separating a small, fast response (thought to be mediated by baroreceptor influences) from a larger, more slowly developing response.


A
BLOOD PRESSURE CS+ RESPONSES
ATROPINE
CHANGE FROM BASELINE
20
10
0
-10
CS+ TONE
0 10 20 30 40 50 60 70 80 90 100 110 120
SECONDS


C
BLOOD PRESSURE CS+ RESPONSES
ATENOLOL
CHANGE FROM BASELINE
20
10
0
-10
CS+ TONE
0 10 20 30 40 50 60 70 80 90 100 110 120
SECONDS


B
HEART RATE CS+ RESPONSES
ATROPINE
CHANGE FROM BASELINE
30
20
10
0
-10
-20
*
CS+ TONE
0 10 20 30 40 50 60 70 80 90 100 110 120
SECONDS

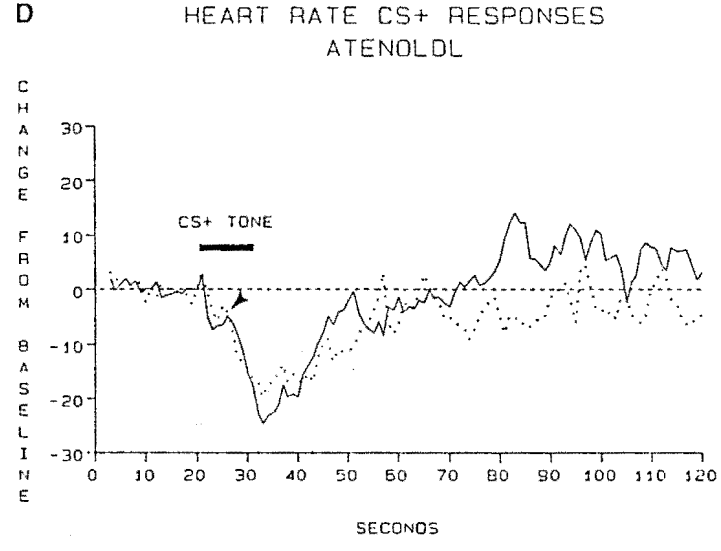
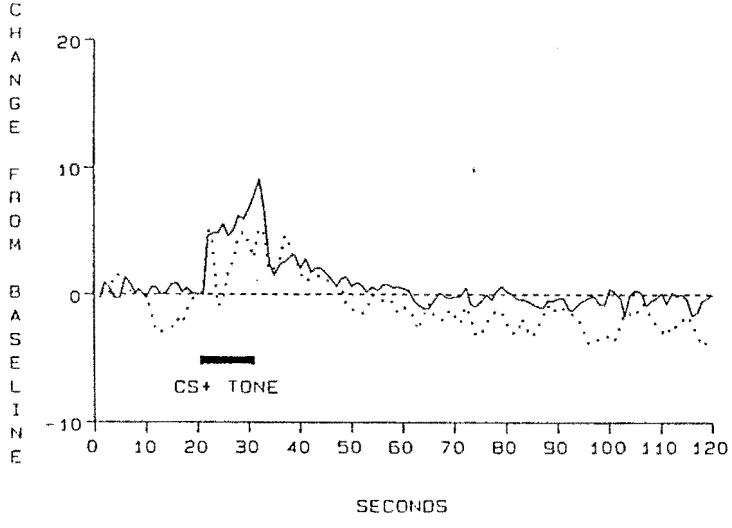

D
HEART RATE CS+ RESPONSES
ATENOLOL
CHANGE FROM BASELINE
30
20
10
0
-10
-20
-30
CS+ TONE
0 10 20 30 40 50 60 70 80 90 100 110 120
SECONDS

Figure 13. Average blood pressure and heart rate responses to the CS+ during combined atropine and atenolol treatment. All responses plotted as change from baseline over time, with baseline computed from seconds 15 to 20. Dotted lines represent MFC lesions, solid lines represent Controls. Bars represent time of CS+ occurrence. A. Mean blood pressure responses. B. Mean heart rate responses.

A

BLOOD PRESSURE CS+ RESPONSES UNDER COMBINED ATROPINE & ATENOLOL TREATMENT



B

HEART RATE CS+ RESPONSES UNDER COMBINED ATROPINE & ATENOLOL TREATMENT

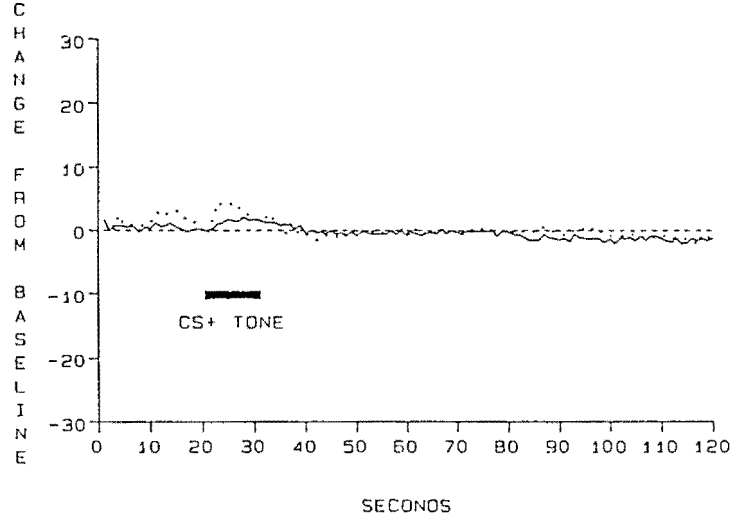


Figure 14. Average blood pressure and heart rate responses to CSr under either atropine or atenolol treatment. All responses plotted as change from baseline over time, with baseline computed from seconds 15 to 20. Dotted lines represent MFC lesions, solid lines represent Controls. Bars represent time of CSr occurrence. A. Blood pressure responses under methyl atropine. B. Heart rate responses under methyl atropine. * denotes MFC response was significantly less than Control. C. Blood pressure responses under atenolol. D. Heart rate responses under atenolol.

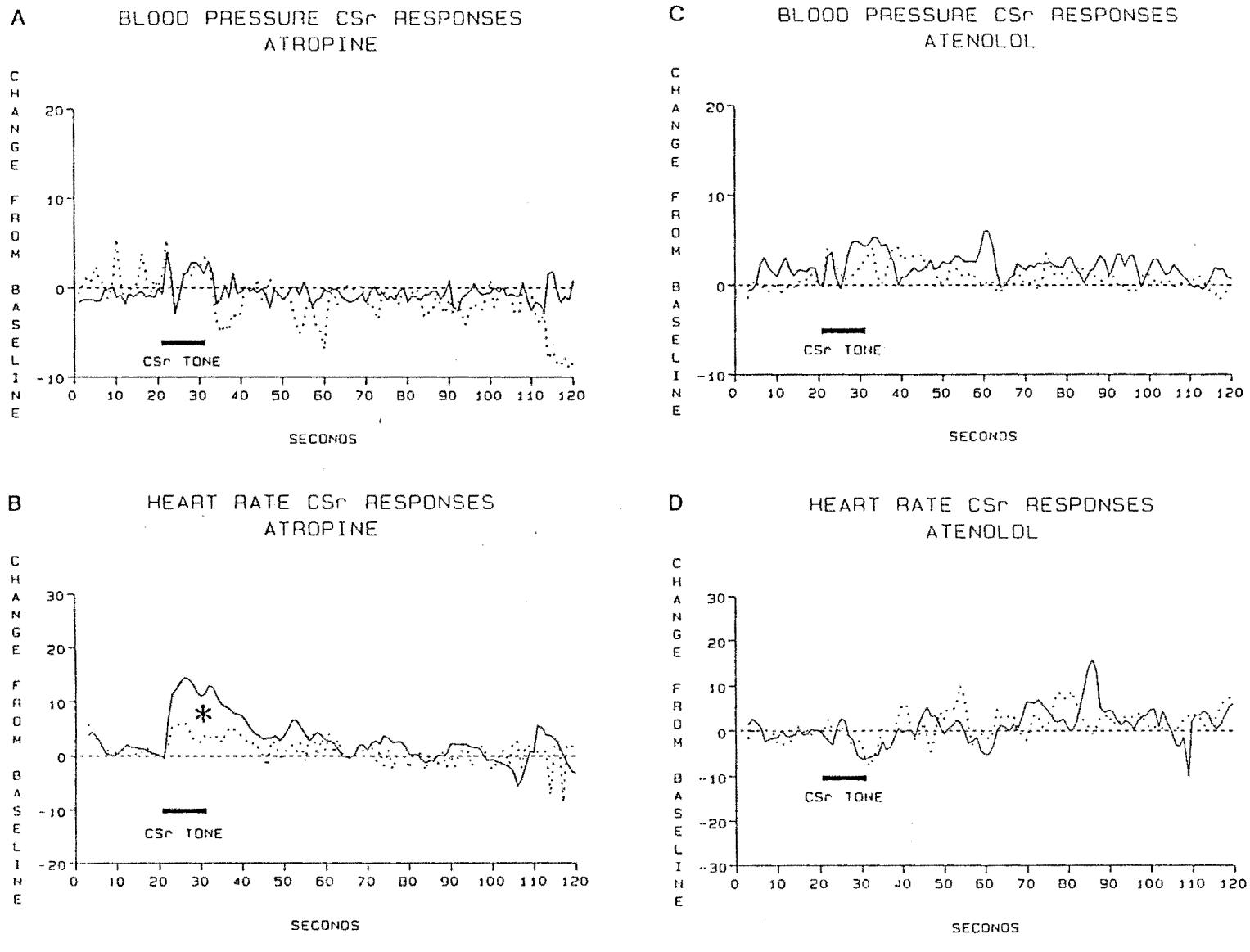
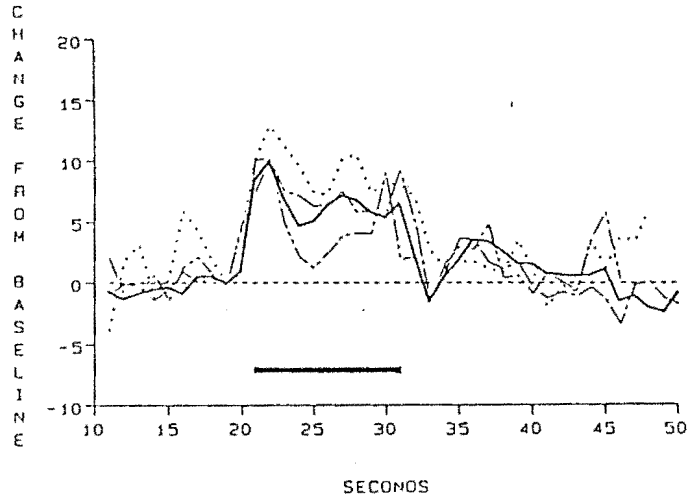

A
BLOOD PRESSURE CSr RESPONSES
ATROPINE
CHANGE FROM BASELINE
20
10
0
-10
CSr TONE
0 10 20 30 40 50 60 70 80 90 100 110 120
SECONDS
C
BLOOD PRESSURE CSr RESPONSES
ATENOLOL
CHANGE FROM BASELINE
20
10
0
-10
CSr TONE
0 10 20 30 40 50 60 70 80 90 100 110 120
SECONDS
B
HEART RATE CSr RESPONSES
ATROPINE
CHANGE FROM BASELINE
30
20
10
0
-10
-20
*
CSr TONE
0 10 20 30 40 50 60 70 80 90 100 110 120
SECONDS
D
HEART RATE CSr RESPONSES
ATENOLOL
CHANGE FROM BASELINE
30
20
10
0
-10
-20
-30
CSr TONE
0 10 20 30 40 50 60 70 80 90 100 110 120
SECONDS

Figure 15. Average blood pressure and heart rate CS+ responses for all groups studied. All responses plotted as change from second 20 versus time. Bar indicates time of CS+ presentation. Solid lines (——) represent combined Controls (N=23); Dotted lines (·····) represent Dorsal+Ventral MFC aspiration lesion responses (N=6); Dot-Dot-Dash lines (··—··) represent Dorsal MFC aspiration lesion responses (N=5); Dot-Dash lines (·-·-·) represent Ventral MFC chemical lesion responses (N=11). A. Blood pressure responses. B. Heart rate responses. * denotes responses significantly different from Control.

A

CONTROL, ASPIRATION AND CHEMICAL LESIONS
BLOOD PRESSURE RESPONSES



B

CONTROL, ASPIRATION AND CHEMICAL LESIONS
HEART RATE RESPONSES

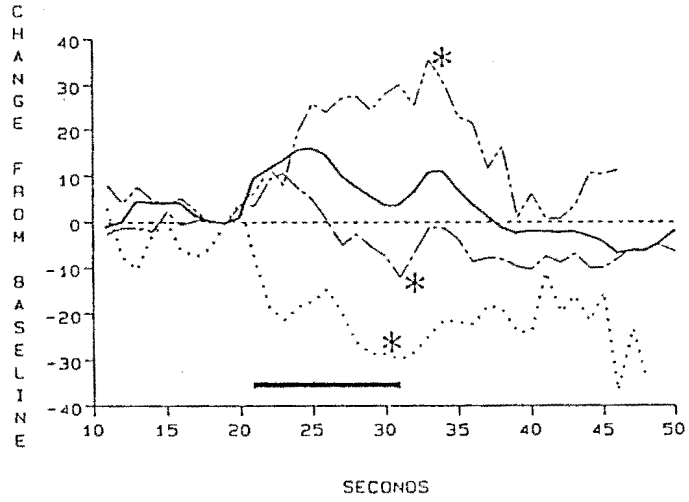
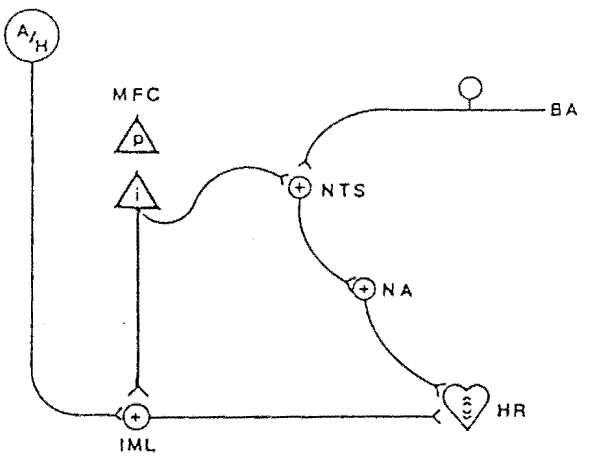
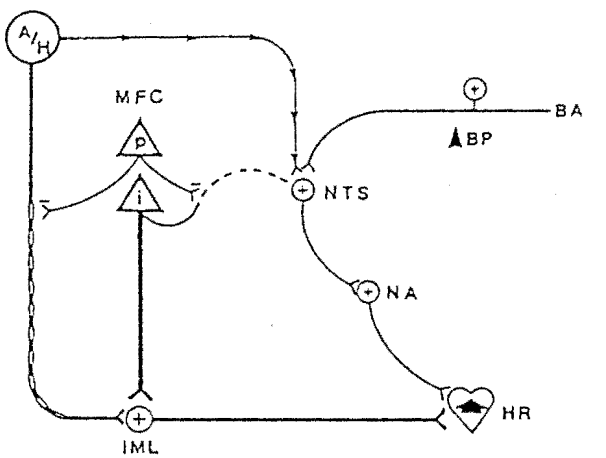


Figure 16. Neuroanatomical representation of possible connections involved in the regulation of cardiovascular CER's. A/H, amygdala and hypothalamus; BA, baroreceptor afferents; p, prelimbic region of MFC; i, infralimbic region of MFC; IML, intermediolateral cell column (sympathetic cell bodies); NA, nucleus ambiguus (parasympathetic cell bodies); NTS, solitary nucleus; "+" denotes excitation of neurons; "-" denotes inhibition of neurons. A. Resting Controls. Sympathetic and parasympathetic influences are "balanced" (≷). B. Controls during CS+ (stress). Sympathetic outflow is dramatically increased due to activation of i and A/H; the prelimbic cortex (p) partially inhibits sympathetic outflow (- - -) and also the baroreceptor influences of i presynaptically. ⟶⟶⟶ denotes slow onset activation (>5 sec) of parasympathetic outflow by other limbic areas (A/H). The net result is a tachycardia (solid arrow) in response to stress. C. MFC lesioned rat's response during CS+ (stress). Loss of the infralimbic region of the MFC (dotted lines) significantly reduces sympathetic outflow, while the slow onset parasympathetic activation is unaffected. The net result is a small initial increase in HR followed by a slow-onset, parasympathetically-mediated bradycardia.

A


A/H
MFC
p
i
BA
NTS
NA
HR
IML

B


A/H
MFC
p
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BP
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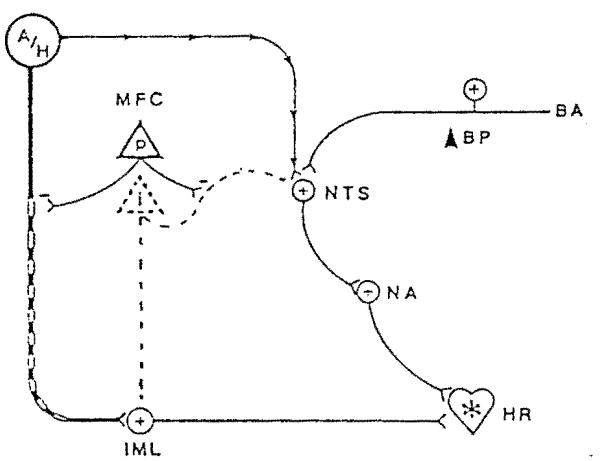
C


A/H
MFC
p
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IML

CHAPTER VI

BARORECEPTOR REFLEX STUDY

INTRODUCTION

The arterial baroreceptor reflex constitutes an important regulatory mechanism of cardiovascular function (Korner, 1979). Neurons in the solitary nucleus, receive information from primary baroreceptor afferents located within the IXth and Xth cranial nerves (Miura & Reis, 1969; Panneton & Loewy, 1980). The activity of these baroreceptor neurons is influenced by higher control mechanisms, such as the hypothalamus, limbic system and cerebral cortex (Hoff et al., 1963; Lofving, 1961; Reis & Cuenod, 1964; Korner et al., 1979). Recent evidence indicates a significant reduction in the resting gain of the baroreceptor reflex following lesions of the medial frontal cortex in rats (Verberne et al., 1987), suggesting that the MFC exerts a tonic influence on the medullary neurons involved in the baroreceptor reflex. Previous neuroanatomical studies in the rat have demonstrated that the medial frontal cortex sends direct projections to the nucleus of the solitary tract (Van der Kooy et al., 1982, 1984; Terreberry & Neafsey, 1983).

The present study determined the gain of the cardiac component of the reflex in both Control and MFC lesioned rats at four timepoints during the CER. The specific aims of this portion of the study were to determine the gain of the parasympathetic limb of the cardiac baroreflex at rest, during the CS+, immediately following the CS+, and during the late phase of the CER trial in Control rats, and to determine whether lesions of the MFC alter the gain of the cardiac baroreflex at any of the timepoints during the CER.

METHODS

The procedures for conditioning, cannula implantation, data recording, animal sacrifice and data analysis are found in the "General Methods" chapter (3) of this dissertation. A description of the chemical MFC lesion procedure can be found in the "Methods" section of chapter 5.

Baroreceptor Reflex Gain

The gain of the baroreflex was assessed during four phases of the CER trial in groups 1 and 2 only. Baroreceptor afferents were activated by transiently raising blood pressure approximately 15 mm Hg with a brief (< 2 sec) inflation and deflation of the balloon catheter, thus evoking a baroreceptor mediated heart rate deceleration. The inflations were mechanically produced by a pump triggered by the computer at the following four points in the analysis:

(1) during the baseline epoch (second 12);

(2) during the CS presentation (second 27);

(3) during the immediate post-CS epoch (second 37);

(4) during the vocalization phase of the post-CS epoch (second 82)

Gain of the reflex was assessed by dividing maximal change in heart rate by the maximal change in blood pressure (dHR/dBP) at each of the four points studied. These ratios were then averaged across animals.

Baroreflex Gain Averages

A full description of the data analysis is given in chapter 3. Briefly, the individual baroreflex gain values for all animals in each group were averaged to produce 4 average baroreflex gain values for each group. After analysis of variance within each group, multiple comparisons were made between CS+ baseline values and corresponding CS, post-CS early, and post-CS late periods; these comparisons then allowed evaluation of changes in baroreflex gain during the various periods of the CER response to the CS+.

The chronological order of the procedures each rat was subjected to during this portion of the study were:

1. MFC lesion/sham - 2 week recovery period
2. Classical conditioning of animal, followed by implantation of cardiovascular and respiratory recording hardware - 24 hour recovery
3. Recording of cardiovascular, respiratory, and ultrasonic vocalization CERs with baroreflex testing
4. Sacrifice by anesthetic overdose
5. Data analysis

Figure 17 displays polygraph tracings of baroreflex trials in Control and MFC lesioned rats during both CS+ and the CSr trials. Inflation of the aortic balloon during the various phases of the trials resulted in increases in BP of about 15 mmHg that were accompanied by decreases in HR. Control CS+ responses (A) show a large heart rate decrease at rest, but the HR responses at the remaining three timepoints are considerably smaller. CSr responses in Controls (B) do not appear to be different across the four timepoints. MFC lesioned (C & D) heart rate responses appear to be permanently reduced during both the CS+ and the CSr trials compared to resting Controls (*).

Figure 18 A & B illustrate the average blood pressure and heart rate responses for the two groups; average blood pressure and heart rate values for the two groups are shown in table 7. There were no significant differences in any of the blood pressure responses, either within or between groups, at any of the timepoints studied. Heart rate responses, however, did show significant differences within the Control group and between the two groups during the resting timepoint (second 12). The resting HR decrease in Controls (-84.3 $\pm$ 10.0 bpm) was significantly larger (*) than the decrease seen in MFC lesioned rats (-37.8 $\pm$ 5.1 bpm). During and immediately following the CS (seconds 27 and 37), Control baroreflex HR responses were significantly less than baseline, indicating inhibition of the baroreflex during stress. At the final timepoint (second 82), the Control HR response was no longer significantly different from baseline, but was significantly greater than the

corresponding MFC response (*). MFC heart rate responses were not significantly different from their baseline response at any of the timepoints studied; all timepoints were significantly less than the resting Control response, indicating a permanent reduction of the baroreflex HR response following MFC lesions.

Figure 18C illustrates the computed baroreflex gain for each individual animal (open symbols) and the corresponding mean gain (filled symbols $\pm$ 1 SEM) at each of the four timepoints for both groups. The mean baroreflex gain values for the four time points are also shown in table 8. The mean baroreflex gain values for MFC lesioned rats did not differ from one another across the four timepoints. Mean Control baroreflex gain values during seconds 27 and 37 were significantly less than the baseline (second 12) gain value. MFC gain values were significantly different from Controls at seconds 12 and 82 (*). During stress, however (seconds 27 and 37), Control and MFC mean baroreflex gain values were nearly identical.

Figure 18D shows that during the CSr, baroreflex gain values within each group did not differ significantly from their respective baseline gain values. Between groups comparisons were significantly different (*) throughout the trial, with the MFC group showing a significantly smaller gain than Controls. Neither of the two groups differed significantly from their respective CS+ resting baroreflex gain.

Cardiac Baroreflex Gain Responses at Rest

In the present study, the gain of the parasympathetic limb of the cardiac baroreflex at rest in Control animals was -5.42 + 0.54 bpm/mmHg, while the CSr resting response was -5.59 + 0.38 bpm/mmHg. In close agreement, Verberne et al. (1987) determined the resting baroreflex gain to be -5.43 + 0.48 bpm/mm Hg, while that reported by Head and McCarty (1987) was -4.12 + 0.24 bpm/mm Hg. Both Verberne et al. and Head & McCarty determined baroreflex gain over a large range of blood pressure values (from 60 to 160 mm Hg) via pharmacological techniques, using phenylephrine hydrochloride to raise blood pressure and sodium nitroprusside to lower it. Phenylephrine is a potent alpha-adrenergic vasoconstrictor and pressor agent which has little influence on the beta-adrenergic receptors of the heart; nitroprusside is a potent vasodilator and hypotensive agent. Both of these drugs also have peripheral effects which may have contributed to the differences observed between our results and those of Head & McCarty.

Verberne et al. (1987), after lesioning the medial prefrontal cortex of the rat using N-Methyl-D-aspartate, reported the gain of the baroreflex to be -3.33 + 0.48 bpm/mm Hg during rest, somewhat larger than our value of -2.46 + 0.30 bpm/mm Hg. Again, this difference may be due to differences in the techniques used to raise the blood pressure, as well as differences in the extent of the incurred lesions.

Cardiac Baroreflex Gain Responses during Stress (CS+)

During stress (CS+), the baroreflex gain is significantly reduced in Control rats. Other studies looking at baroreceptor gain during stimulation of the hypothalamus, amygdala, and during presentation of natural stressors have reported similar reductions in the gain of the baroreflex during stress (Schlor et al., 1984; Pardini et al., 1986). Our data indicate that the reduction in the gain persists after the CS+, since 5 seconds after the cessation of the CS+, the baroreflex gain (-2.34) is nearly identical to that found during the CS+ (-2.31). The variability between animals during stress, as illustrated by the plots of the individual animal gains in figure 2C, is very small at the two time points in question (seconds 27 and 37). By second 82, however, the mean baroreflex gain has begun to return to pre-stress levels, possibly indicating a return to a more "relaxed" emotional state in the animal and the resetting of the baroreflex gain to prestress levels.

MFC lesioned rats were unable to alter or reduce the gain of the baroreflex further in response to stress, since none of the tests of baroreflex gain in MFC lesioned rats during the various phases of the CER resulted in gain values which were significantly different than that seen during rest. The baroreflex had, in a sense, "bottomed out." The loss of MFC neurons resulted in a permanent reduction in the gain of the baroreflex, indicating that this cortical area plays an important role in the modulation of the cardiac baroreflex gain during stress.

Cardiac Baroreflex Gain Responses during the CSr

Baroreflex gain was not reduced in response to the CSr at any point for either group compared to resting CS+ values, implying that the "emotional state" of the rat was not significantly altered by the CSr tone. The gain of the baroreflex in MFC lesioned rats was, however, significantly reduced compared to Controls, again indicating that MFC lesions permanently altered the gain of the baroreflex.

Figure 19 illustrates a possible neuroanatomical explanation for the results seen during baroreflex testing. The MFC may be directly modulating the gain of the baroreceptor reflex arc at rest, presumably at the level of the NTS. During stress, the MFC efferents to the NTS are inhibited, resulting in a net decrease in parasympathetic outflow and a reduction in the gain of the baroreflex. During the CSr, the animal is not "stressed," and the neurons inhibiting the MFC efferents are not activated, thereby allowing the MFC to modulate the baroreceptor reflex arc as they did at rest, resulting in no net loss of parasympathetic outflow or reduction of baroreflex gain. Following MFC lesions, there is no modulation of the baroreflex centers in the NTS, resulting in permanent reduction in the cardiac baroreflex gain.

CONCLUSIONS

The normal baroreceptor reflex gain was significantly reduced during and immediately following the CS+ in Controls, presumably by inhibition of MFC neurons which normally facilitate the reflex during "non-stress" states. This reduction in gain during the CS+, therefore, was directly related to the emotional state of the animal. By the fourth test of the baroreflex gain at 82 seconds (one minute after CS+ onset), the gain has returned to near baseline levels, indicating a return to a non-stress state and a decrease in the amount of inhibition of MFC efferents projecting to the baroreflex afferents. Medial frontal cortex lesions permanently and significantly reduced the baroreceptor reflex gain, and no additional reduction in gain could be induced by stress.

CSr responses did not show any differences between baseline and CER tests or from the resting CS+ response, indicating that the CSr trial was not considered a "stressful" event by the animals. MFC CSr responses were significantly different from Controls at all time points studied, again indicating a permanent reduction in baroreflex gain following MFC lesions.

The medial frontal cortex, therefore, is an important cortical structure in the regulation of the parasympathetic limb of the cardiac baroreflex gain.

Figure 17. Polygraph tracings of Control and MFC lesioned CS+ baroreflex trials illustrating ultrasonic vocalizations (USV), blood pressure (BP) and heart rate (HR). Bar = 10 seconds; balloon inflations causing rise in BP indicated by small arrows. * denotes Control resting baroreflex-mediated heart rate response to a 15 mmHg increase in blood pressure. Blood pressure increases caused by inflation of the intra-aortic balloon catheter are indicated by small arrows. A. Control CS+ responses. B. Control CSr responses. C. MFC CS+ responses. D. MFC CSr responses.

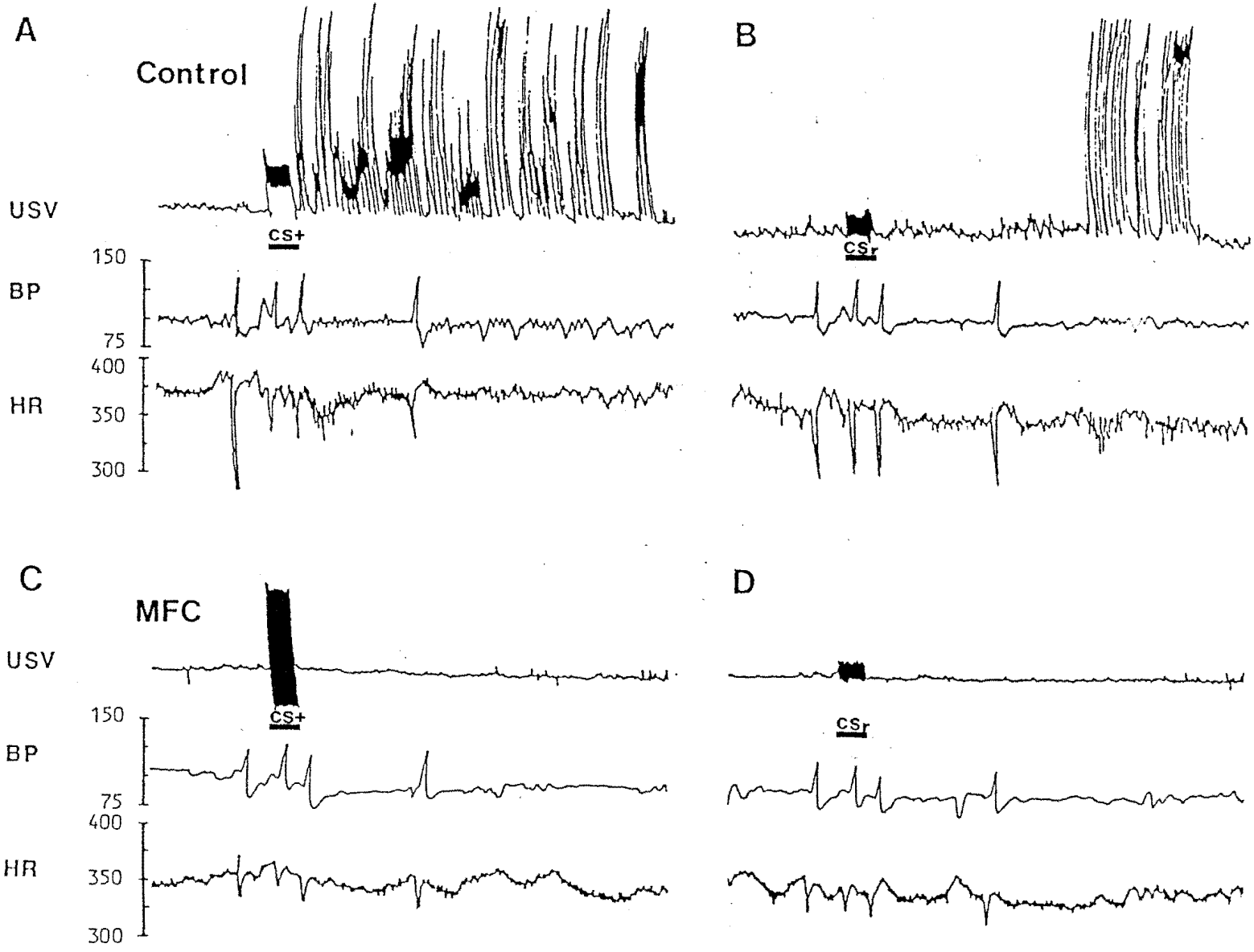
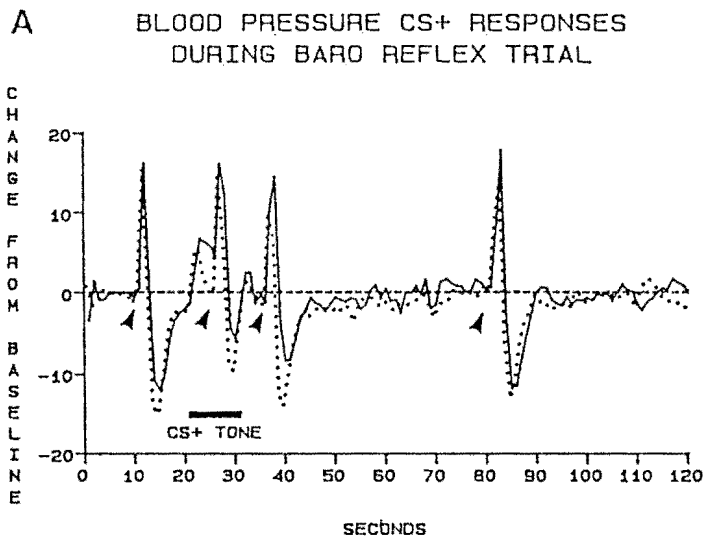
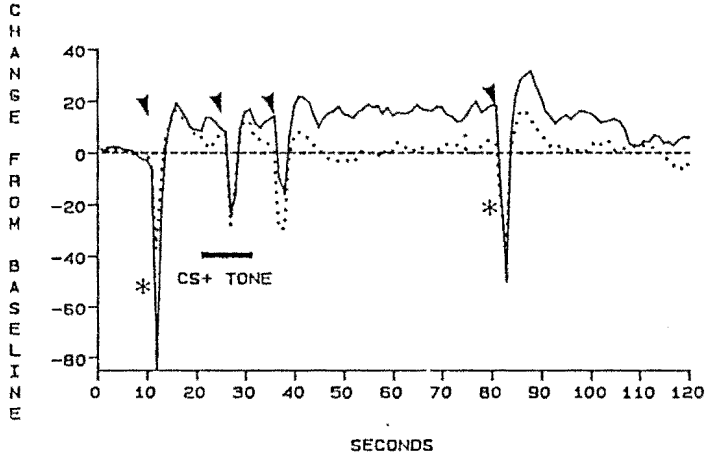
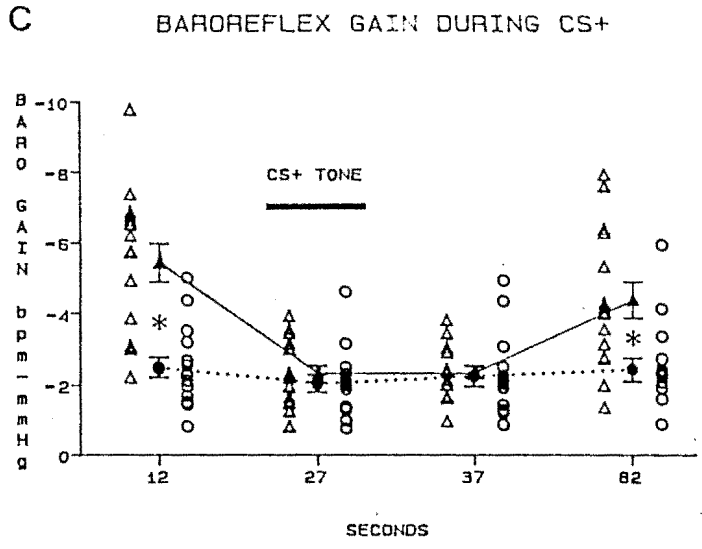

A
Control
USV
CS+
150
BP
75
400
HR
350
300
B
CSr
C
MFC
USV
CS+
150
BP
75
400
HR
350
300
D
CSr

Figure 18. A & B. Mean blood pressure (A) and heart rate (B) tracings and baroreflex gain changes for Control and MFC lesioned animals. Solid lines represent Control responses; dotted lines represent MFC lesioned responses; bar represents CS+ presentation. Responses plotted as change from baseline vs. time. Timepoints of mechanically induced increases of blood pressure (balloon inflation) are indicated by arrows. * denotes significant differences ($p<0.01$) between the two groups. C & D. Baroreflex gain changes during CS+ (C) and CSr (D) trials, plotted as bpm/mmHg vs. time. Open triangles represent individual Control responses (N = 13); filled triangles represent **mean** Control responses for each timepoint $\pm$ 1 SEM. Open circles represent individual MFC lesion responses (N = 11); filled circles represent **mean** MFC lesion responses for each timepoint $\pm$ 1 SEM. * denotes significant differences ($p<0.01$) between the two groups.


A
BLOOD PRESSURE CS+ RESPONSES
DURING BARO REFLEX TRIAL
CHANGE FROM BASELINE
20
10
0
-10
-20
CS+ TONE
0 10 20 30 40 50 60 70 80 90 100 110 120
SECONDS


B
HEART RATE CS+ RESPONSES
DURING BARO REFLEX TRIAL
CHANGE FROM BASELINE
40
20
0
-20
-40
-60
-80
CS+ TONE
0 10 20 30 40 50 60 70 80 90 100 110 120
SECONDS


C
BAROREFLEX GAIN DURING CS+
BARO GAIN bpm/mmHg
-10
-8
-6
-4
-2
0
CS+ TONE
12 27 37 82
SECONDS

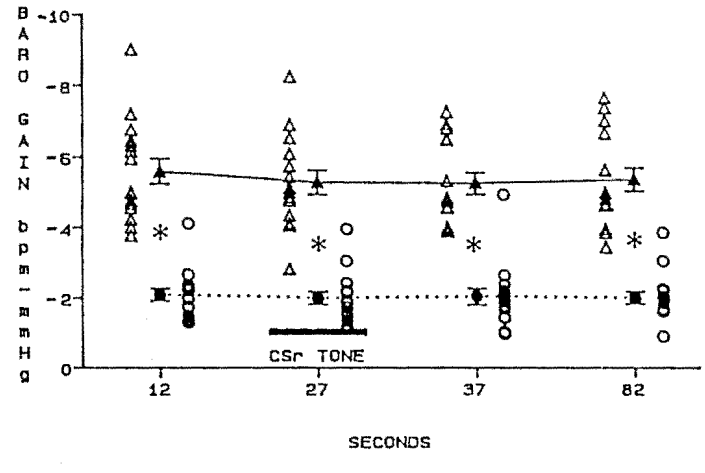

D
BAROREFLEX GAIN DURING CSr
BARO GAIN bpm/mmHg
-10
-8
-6
-4
-2
0
CSr TONE
12 27 37 82
SECONDS

Table 7. **MEAN BLOOD PRESSURE AND HEART RATE RESPONSES DURING CARDIAC BAROREFLEX TESTING**

BLOOD PRESSURE RESPONSES

SUBJECT	SECOND 12	SECOND 27	SECOND 37	SECOND 82
CS+ RESPONSES				
Control	16.3 ± 2.0	16.2 ± 2.5	16.0 ± 2.7	16.3 ± 2.1
MFC	15.6 ± 3.4	15.1 ± 2.1	13.8 ± 2.0	14.3 ± 3.8
CSr RESPONSES				
Control	16.2 ± 1.9	16.4 ± 2.0	16.2 ± 1.9	15.9 ± 2.3
MFC	15.9 ± 2.6	15.5 ± 3.1	16.0 ± 2.2	15.7 ± 3.0

HEART RATE RESPONSES

CS+ RESPONSES				
Control	-84.3 ± 10.0	-31.3 ± 7.5*	-31.0 ± 7.1*	-67.8 ± 8.4
MFC	-37.8 ± 5.1#	-29.3 ± 7.3	-32.3 ± 5.9	-35.2 ± 10.0#
CSr RESPONSES				
Control	-89.4 ± 5.9	-84.5 ± 5.4	-84.0 ± 5.0	-85.8 ± 5.1
MFC	-33.6 ± 3.2#	-31.8 ± 3.3#	-32.5 ± 3.8#	-32.0 ± 3.3#

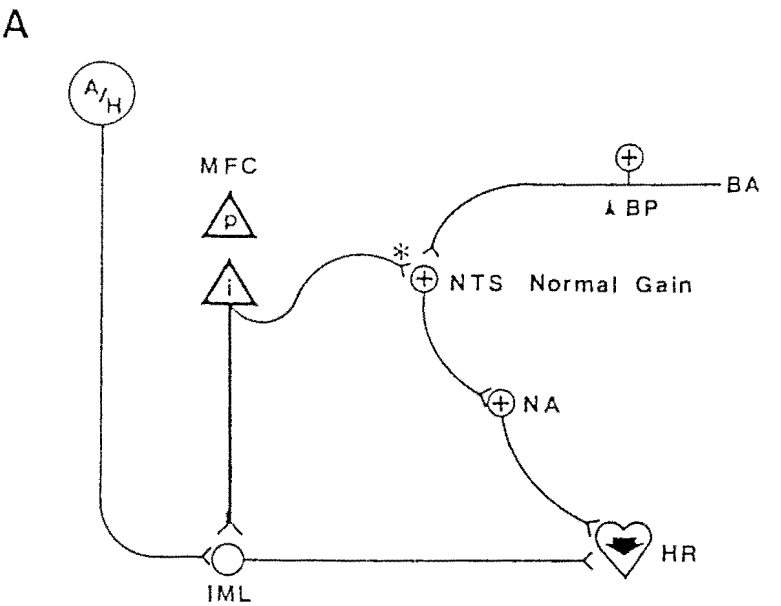
Mean blood pressure and heart rate baroreflex responses during the CS+ and CSr trials for Control and MFC lesioned rats. Responses reported as mean ± 1 SEM. * denotes responses significantly different from **baseline** trial (SECOND 12; $p<0.05$); # denotes responses significantly different ($p<0.01$) **between** groups.

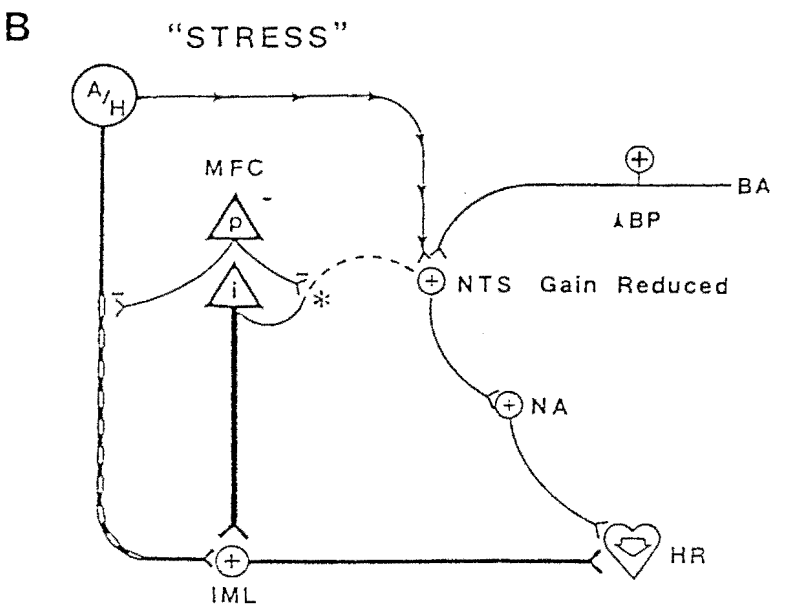
Table 8. **CS+ AND CSr MEAN BAROREFLEX GAIN RESPONSES**

SUBJECT	SECOND 12	SECOND 27	SECOND 37	SECOND 82
CS+ RESPONSES				
Control	-5.42 ± 0.54 #	-2.31 ± 0.24*	-2.31 ± 0.19*	-4.38 ± 0.51 #
MFC	-2.46 ± 0.30	-2.02 ± 0.25	-2.22 ± 0.30	-2.40 ± 0.34
CSr RESPONSES				
Control	-5.59 ± 0.37 #	-5.28 ± 0.34 #	-5.25 ± 0.31 #	-5.36 ± 0.34 #
MFC	-2.09 ± 0.18	-1.99 ± 0.19	-2.03 ± 0.24	-1.99 ± 0.20

Mean baroreflex gain values for Control and MFC lesioned groups during the CS+ and CSr presentations. Values reported as mean gain in bpm/mmHg ± 1 SEM. * denotes responses significantly different from **baseline** trial (SECOND 12; $p<0.05$); # denotes responses significantly different **between** groups ($p<0.01$).

Figure 19. Neuroanatomical representation of possible connections involved in the regulation of cardiovascular CER's during the baroreflex. A. Resting Controls. Increases in blood pressure (▲ BP) stimulate baroreceptor afferents (BA) which project to the solitary nucleus (NTS). Excitatory projections from the infralimbic MFC (i) synapse in the NTS and help to maintain the baroreflex gain (*). Via NTS projections to the nucleus ambiguus (NA), heart rate is decreased in response to BP increases. B. Controls during stress. Stress results in activation of various central nervous system "autonomic" areas, such as the amygdala and hypothalamus (A/H) and the prelimbic (p) and infralimbic MFC. The projections from the infralimbic MFC to the NTS are inhibited pre-synaptically (*), possibly by the prelimbic MFC, resulting in reduction of the baroreflex gain at the NTS. Slow onset activation of the parasympathetic nervous system occurs via direct activation by other limbic areas (such as the A/H) (→→→→), and may aid in returning the baroreflex to "pre-stress" levels.


A
A/H
MFC
p
i
BA
BP
NTS Normal Gain
NA
HR
IML


B
"STRESS"
A/H
MFC
p
i
BA
BP
NTS Gain Reduced
NA
HR
IML

CHAPTER VII

SINOAORTIC DENERVATION RESPONSES

INTRODUCTION

The relative contributions of sympathetic and parasympathetic output to the baroreceptor reflex and the function of the baroreflexes in conscious and anesthetized animals has been examined in several species, including dogs (Berkowitz et al., 1969; Cowley et al., 1972, 1980; Cox & Bagshaw, 1980; Thames & Kontos, 1970; Vatner et al., 1974), rabbits (Guo et al., 1982; Stinnett et al., 1981) and rats (Coleman, 1980; Norman et al., 1981; Stornetta et al., 1987; Trapani et al., 1986; Alper et al., 1987; Head & McCarty, 1987). The bradycardia associated with the early or "fast" component of the baroreflex was determined to be wholly parasympathetic (Coleman, 1980). However in cases of chronic hypertension or sustained activation of the baroreflexes, sympathetic influences, specifically the beta-adrenergic component, over-ride or replace the short term parasympathetic response (Coleman, 1980; Alper et al., 1987; Stornetta et al., 1987). The rapid vagal control of heart rate during chronic baroreceptor activation is therefore temporary; it is replaced in time by the more slowly developing sympathetic influences (Coleman, 1980). Sympathetic output, therefore, appears to be the dominant component regulating long-term vasomotor and cardiac variability. The increases in heart rate and blood pressure variability following sinoaortic denervation are produced by fluctuations in the sympathetic vasomotor tone and cardiac tone (Trapani et al., 1986).

In order to determine to what extent the baroreceptors contribute to the cardiovascular CER pattern in rats, sinoaortic denervation (SAD) of the

baroreceptor afferents was performed in both Control and MFC lesioned groups. Sinoaortic denervation does not prevent classical conditioning in rabbits, but no known studies have been performed in the rat.

The specific aims of this portion of the study were to determine the contribution of the baroreceptor reflex during the CER by comparing the CS+ responses in animals which had undergone sinoaortic denervation (SAD lesioned animals) to the CS+ responses in baroreceptor intact Controls, and to determine the effects of MFC lesions independent of baroreceptor activation by comparing the CS+ responses in SAD+MFC lesioned animals to the CS+ responses in baroreceptor intact MFC lesioned animals. CSr responses were also compared.

METHODS

The procedures for classical conditioning, cannula implantation, recording of data, animal sacrifice and data analysis are found in the "General Methods" chapter (4) of this dissertation. A description of the chemical MFC lesion procedure can be found in the "Methods" section of chapter 5.

Sinoaortic Denervation

Those animals receiving sinoaortic denervation lesions were anesthetized with ketamine HCl (100 mg/kg) and restrained in a supine position on a rat board. A midline incision was made in the neck, and the hyoid muscles retracted to gain access to the baroreceptor afferent nerves. The vagus nerves and the carotid arteries were carefully removed from the neurovascular sheaths and sympathetic trunks; the sheaths and the sympathetic trunks were then cut bilaterally and the superior cervical ganglion removed. The superior laryngeal nerves were sectioned bilaterally near the vagi. Finally, the carotid sinuses were denervated by stripping the region of the bifurcation of all extraneous fibers and adventitia, followed by application of 10% phenol (in 95% ethanol) to the internal, external, and common carotid arteries as far as the thyroid artery, avoiding the vagi (Krieger, 1964). All wounds were then sutured and swabbed with bactericidal soap. The animal was returned to his home cage and allowed to recover for two weeks. Sham surgery consisted of a midline neck incision and bilateral dissection and retraction of the hyoid muscles (Alper et al., 1987). The efficacy of the lesions was tested prior to

CER testing by viewing the HR response during either inflation of the aortic balloon catheter (see Cannula Implantation) or following a bolus phenylephrine injection. Only changes in heart rate of less then 20 beats per minute during a 50 mmHg increase in BP were utilized in this study. This value was chosen prior to data analysis, and is generally considered to be a good indication of a successful denervation (Alper et al., 1987), since the HR decrease in intact animals to the same stimulus is greater than 80 beats per minute. Changes in heart rate for the animals used in this study averaged only 6.1 $\pm$ 2.11 bpm following a 50 mmHg increase in blood pressure. In those animals in which denervation was not successful (heart rate changes > 20 bpm), the animal was not used in the data analysis.

The chronological order of the procedures each rat was subjected to during this portion of the study were:

1. SAD lesion/sham - 2 week recovery period
2. MFC lesion/sham - 2 week recovery period
3. Classical conditioning of animal, followed by implantation of cardiovascular and respiratory recording hardware - 24 hour recovery
4. Recording of cardiovascular, respiratory, and ultrasonic vocalization CERs
5. Sacrifice by anesthetic overdose
6. Data analysis

Comparison of Resting Blood Pressure and Heart Rate

Mean resting values for both blood pressure and heart rate measured during a 2 minute period four weeks after the SAD surgery were not significantly altered by sinoaortic denervation. Table 9 displays the blood pressure and heart rate for baroreceptor-intact Controls and SAD lesioned animals during this 2 minute resting trial. Variability, determined as the mean standard deviation of individual animals within each group during the CER trials was, however, significantly increased in SAD lesioned rats for both blood pressure and heart rate when compared to baroreceptor intact animals during the 2 minute resting period.

SAD CS+ Responses

Cardiovascular CS+ responses for SAD and SAD + MFC lesioned animals are shown in figure 20 and table 10. The cardiovascular CS+ response for SAD and SAD + MFC lesioned animals consisted of a large pressor response coupled with a large tachycardia. The pressor response was bimodal, with a small peak occurring initially and the principal peak occurring at the end of the CS+. Blood pressure slowly returned to baseline following the CS+ in SAD rats, but quickly returned to baseline in SAD + MFC rats. Heart rate remained elevated during and immediately following the CS+ in the SAD group, but returned temporarily to baseline in the SAD + MFC group.

Late cardiovascular CS+ responses in SAD lesioned animals showed blood pressure returning to baseline by second 50, with variability significantly increased ($p<0.05$) compared to baseline (Table 11). Mean blood pressure during the late CER phase in SAD + MFC lesioned animals was significantly greater than both baseline and SAD lesioned animals. Blood pressure variability was also significantly increased during the late phase compared to baseline. Mean heart rate remained significantly elevated (17.90 $\pm$ 7.27 bpm) above baseline throughout the remainder of the trial in SAD rats, and variability was also significantly increased compared to baseline. Heart rate in SAD + MFC rats, after a brief return to baseline at second 40, increased significantly compared to baseline to a mean of 30.60 $\pm$ 9.56 bpm during the late phase of the CS+ response. This increase was also significantly greater than that of SAD lesioned animals. Variability was significantly increased compared to baseline.

SAD CSr Responses

The cardiovascular CSr responses (Figures 20) for SAD and SAD + MFC lesioned animals consisted of a pressor response paired with a small tachycardia. Blood pressure slowly returned to baseline following the CSr in both groups. The mean heart rate response for both groups was significantly smaller than their corresponding CS+ responses. Heart rate returned to baseline shortly after the CSr.

The late cardiovascular CSr responses were generally smaller in amplitude than the CS+ responses. Mean blood pressure was not significantly different from baseline, but variability was significantly increased compared to baseline (Table 11). Variability during the late CSr phase was significantly less than the CS+ late BP response. Mean HR was not significantly different from baseline throughout most of the late phase of the trial for both groups, except during the very late phases of the SAD + MFC trial, where movement occurred. Variability was not significantly different from baseline or from corresponding CS+ responses for either group.

DISCUSSION

The removal of baroreceptor input to the central nervous system results in a large, transient rise in arterial pressure (Krieger, 1967; Reis & Talman, 1984). This temporary pressor effect is produced by bilateral lesions of the solitary nucleus (site of termination of the baroreceptor afferents) or by denervation of the baroreceptor afferents (Nathan & Reis, 1977; Krieger, 1964), due to the removal of tonic inhibitory influences on sympathoexcitatory centers (Brody et al., 1984; Alper et al., 1987). The long-term effects of sinoaortic denervation (SAD) remain somewhat controversial in regards to whether or not the observed hypertension is permanent (Cowley et al., 1972; Ferrario et al., 1969; Ito & Scher, 1981; Krieger, 1967; Trapani et al., 1986). There is complete agreement, however, that removal of the primary baroreceptor afferents causes increased variability in mean arterial pressure in most species (Ferrario et al., 1969; Krieger, 1967; Korner et al., 1984; Alper et al., 1987; Trapani et al., 1986).

Resting blood pressure and heart rate values (Table 9) in SAD lesioned rats were not significantly different from baroreceptor-intact animals during the 2 minute resting trial; variability, however, was significantly increased. These findings agree with those of Alper et al. (1987) and others (Buchholz & Nathan, 1984; Cowley et al., 1972, 1980). According to Cowley (1980), "Excitement or arousal caused by visual, auditory and olfactory stimuli, anticipation of feeding, grooming, REM sleep, pain, etc. was associated with dramatic elevations of arterial pressure." Classical conditioning results in a definite excitatory or arousal condition in the rat,

and therefore increased variability in MAP and heart rate should be noted during and following the CS. During all phases of the CER, SAD lesioned animals showed significantly greater variability than corresponding baroreceptor-intact animals (Table 11). SAD lesions did not prevent conditioning of cardiovascular parameters in rats, which is in agreement with previous studies in rabbits (Jarrell et al., 1986).

Baroreceptor Contributions to the CS+ in SAD Lesioned Rats

The sympathetic nervous system is responsible for the maintenance (and possibly the generation of) the increased variability in SAD lesioned animals (Trapani et al., 1986; Alper et al., 1987), implying that a fairly high sympathetic tone exists following SAD (Coleman, 1980; Alper et al., 1987). This increased sympathetic activation, coupled with a lack of baroreceptor mediated parasympathetic activation due to denervation of the baroreceptor afferents, results in a large net increase in blood pressure and heart rate in response to the CS+ in SAD lesioned rats. During the CER in both baroreceptor-intact Controls and SAD lesioned rats, the early response to the CS+ was a pressor response paired with a tachycardia. However, the SAD lesioned blood pressure and heart rate responses were significantly greater than those of baroreceptor-intact Controls. The tachycardia in Controls was due to a dominant sympathetic activation (Chapter 5). The present results agree with these findings.

Figure 21 illustrates one possible neuroanatomical explanation for the results seen in baroreceptor-intact and SAD lesioned animals. In intact Controls at rest,

the baroreceptor afferents respond to acute increases in blood pressure by increasing parasympathetic activation to the heart, thereby slowing it. After denervation of the baroreceptor afferents, acute changes in blood pressure are no longer reflexively controlled. Under stress (CS+), activation of the sympathetic nervous system occurs through several routes. In intact Controls, although the gain of the baroreflex has been reduced, some baroreflex-mediated parasympathetic outflow does occur. The net response in intact Controls to this coactivation of the sympathetic and parasympathetic nervous systems is a pressor response paired with a tachycardia. Following SAD, the parasympathetic component was almost entirely lost, while sympathetic tone was not directly affected. The net response to the CS+ was a large sympathetic outflow which was no longer buffered by baroreflex-mediated parasympathetic activation, yielding a much larger tachycardia than that seen in baroreceptor-intact Controls. The contribution of the baroreceptor reflex during the CS+, therefore, is to buffer/reduce the sympathetically mediated cardiovascular responses. Without the baroreceptor reflex, CER responses are "unchecked," resulting in a significantly larger tachycardia, as well as increased cardiovascular variability.

Baroreceptor Contributions to the CS+ Following MFC Lesions

During the CER in MFC lesioned groups (MFC & SAD+MFC), the blood pressure responses in MFC lesioned rats were similar to that of MFC-intact animals. Heart rate responses, however, were significantly different. SAD+MFC

lesioned rats responded to the CS+ with a large tachycardia, while MFC lesioned rats responded to the CS+ with a brief tachycardia followed by a slow-onset bradycardia. Pharmacological evidence from the MFC group (Chapter 5) indicated a 52 % reduction in sympathetic outflow during the CS compared to Controls, and a permanent reduction in the gain of the baroreflex (Chapter 6). The reduction in sympathetic outflow in MFC lesioned rats was sufficient to shift the dominance of the autonomic outflow to the parasympathetic component, resulting in the bradycardia. Denervation of the baroreceptor afferents in the SAD+MFC group significantly reduced parasympathetic activation during the CS+, thereby resulting in the sympathetic component once again being dominant and producing the observed tachycardia.

Figure 22 illustrates one possible neuroanatomical explanation of the responses seen in MFC and SAD+MFC lesioned rats. Lesions of the MFC result in the loss of neurons which activate the sympathetic nervous system during the CS+ (stress). In baroreceptor-intact MFC lesioned rats, the CS+ resulted in a pressor response which reflexively activated the baroreflex, resulting in increased parasympathetic outflow. Coupled with the decrease in sympathetic outflow due to the loss of MFC neurons directly activating the sympathetic system and the inhibition of limbic sympathoexcitatory neurons by the prelimbic MFC (Al Maskati & Zbrozyna, 1989), the net heart rate response was a parasympathetically mediated, slow-onset bradycardia. Denervation of baroreceptor afferents results in a significant loss of parasympathetic outflow. Consequently, the sympathetic

component again becomes dominant during the CER.

Baroreceptor Contributions to CSr Responses

The SAD lesioned and SAD+MFC CSr cardiovascular response patterns were similar to the CS+ responses, namely a pressor response paired with a tachycardia. The CSr HR responses were significantly smaller, however, than those seen in the CS+ responses, apparently due to decreased sympathetic activation during the CSr (described earlier in Chapter 5), which was accompanied by little or no parasympathetic activation. These responses agree with those described earlier in Chapter 5, which concluded that the CSr was mediated by sympathetic activation only.

CONCLUSIONS

SAD lesioned rats respond to the CS+ with a pressor response paired with a large tachycardia. These responses appeared to be mediated primarily by sympathetic outflow. The tachycardia was greater in amplitude, duration and variability compared to baroreceptor-intact Controls. This implies that the baroreceptor reflex normally acts to buffer the sympathetic outflow in Controls and helps reduce variability.

SAD+MFC rats also responded to the CS+ with a pressor response paired with a large tachycardia; the heart rate response was significantly different than MFC lesioned rats. The CS+ response in SAD+MFC lesioned animals appeared to be mediated primarily by sympathetic activation, which was significantly different than the parasympathetically mediated CS+ response seen in baroreceptor-intact MFC lesioned animals.

The loss of the parasympathetically-mediated baroreceptor component reduced the central nervous system's ability to "buffer" blood pressure and heart rate responses, and increased variability for both SAD lesioned and SAD+MFC groups.

Table 9. **RESTING MEAN BLOOD PRESSURE, HEART RATE, AND VARIABILITY**

RESTING RESPONSE	CONTROL (N = 13)	SAD (N = 5)
Blood Pressure	107.2 ± 2.5	108.1 ± 9.4
Blood Pressure Variability	9.0 ± 1.4	20.7 ± 3.2*
Heart Rate	364.4 ± 5.0	368.7 ± 16.2
Heart Rate Variability	17.7 ± 2.3	34.9 ± 4.7*

Mean blood pressure and heart rate responses and associated variability during the 2 minute resting trial ± 1 standard error. Mean responses were calculated by averaging the blood pressure and heart rate responses during the two minute resting baseline trial taken before CER testing for Controls and SAD lesioned animals. Variability was determined by averaging the mean standard deviation value for individual animals in each group. * denotes SAD responses significantly different from corresponding Control response.

Figure 20. Blood pressure and heart rate CS+ and CSr responses following sinoaortic denervation of the baroreceptor afferents. **Heavy solid lines** represent **SAD** lesioned rats; **heavy dotted lines** represent **SAD+MFC** lesioned animals; light solid lines represent sham-operated Controls; light dotted lines represent MFC lesioned rats. Bar represents occurrence of CS+ or CSr tone. A. Blood pressure CS+ responses. B. Heart rate CS+ responses. C. Blood pressure CSr responses. D. Heart rate CSr responses.

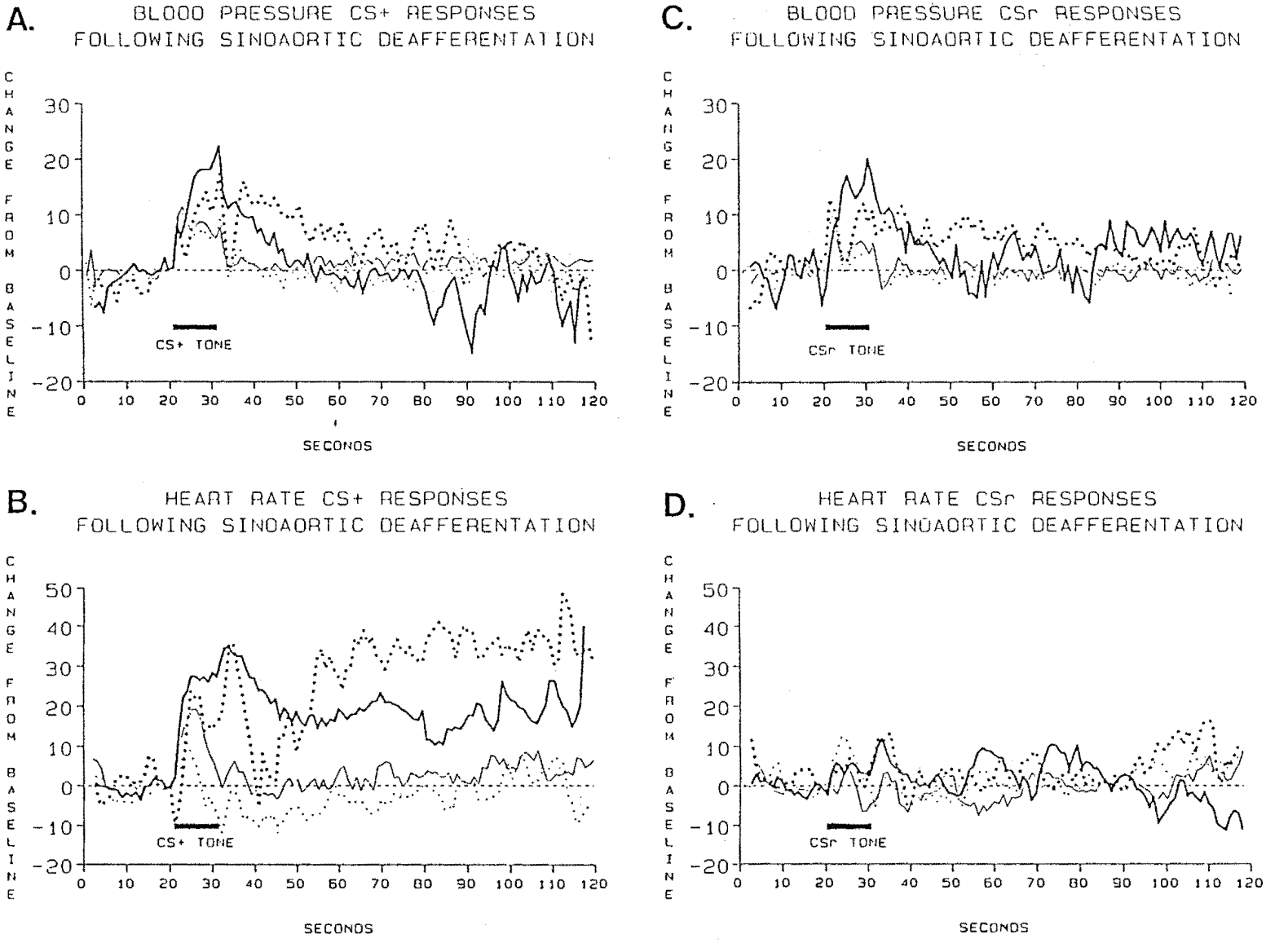

A.
BLOOD PRESSURE CS+ RESPONSES
FOLLOWING SINOAORTIC DEAFFERENTATION
CHANGE FROM BASELINE
30
20
10
0
-10
-20
CS+ TONE
0 10 20 30 40 50 60 70 80 90 100 110 120
SECONDS
C.
BLOOD PRESSURE CSr RESPONSES
FOLLOWING SINOAORTIC DEAFFERENTATION
CHANGE FROM BASELINE
30
20
10
0
-10
-20
CSr TONE
0 10 20 30 40 50 60 70 80 90 100 110 120
SECONDS
B.
HEART RATE CS+ RESPONSES
FOLLOWING SINOAORTIC DEAFFERENTATION
CHANGE FROM BASELINE
50
40
30
20
10
0
-10
-20
CS+ TONE
0 10 20 30 40 50 60 70 80 90 100 110 120
SECONDS
D.
HEART RATE CSr RESPONSES
FOLLOWING SINOAORTIC DEAFFERENTATION
CHANGE FROM BASELINE
50
40
30
20
10
0
-10
-20
CSr TONE
0 10 20 30 40 50 60 70 80 90 100 110 120
SECONDS

Table 10. **MEAN CARDIOVASCULAR CER RESPONSES**

	BLOOD PRESSURE RESPONSES		HEART RATE RESPONSES	
GROUP (N)	BASELINE	CS	BASELINE	CS
		CS+ RESPONSES		
SAD (5)	0.1 ± 1.2	13.3 ± 3.7*	-0.6 ± 2.5	23.0 ± 8.2*
SAD+MFC (4)	-1.7 ± 1.3	8.4 ± 6.7	2.3 ± 3.4	12.1 ± 5.4
		CSr RESPONSES		
SAD (5)	2.3 ± 4.4	11.3 ± 4.9	-0.3 ± 3.1	7.0 ± 2.1*
SAD+MFC (4)	-0.8 ± 0.4	3.5 ± 6.2	2.5 ± 1.6	9.1 ± 6.4

Mean blood pressure and heart rate responses during the various phases of the CER ± 1 SEM. All responses are reported as changes from second 20. Baseline is defined as the mean of seconds 9 to 19; CS is defined as the mean of seconds 21-31. * denotes responses significantly different from baseline ($p<0.05$).

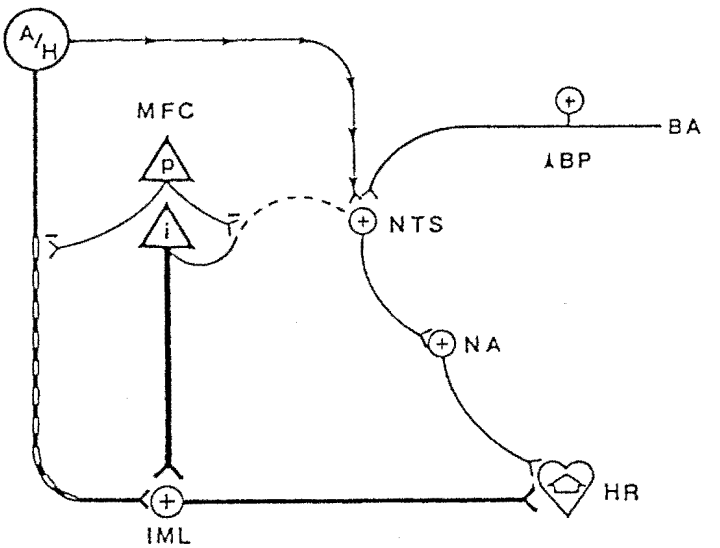
Table 11. **CARDIOVASCULAR VARIABILITY MEASUREMENTS**

	BLOOD PRESSURE RESPONSES			HEART RATE RESPONSES		
GROUP	BASELINE	CS	LATE	BASELINE	CS	LATE
			CS+ RESPONSES			
Control	2.4 ± 0.5	5.4 ± 0.9*	3.3 ± 0.5 #	4.1 ± 1.1	10.0 ± 1.2*	9.9 ± 1.3*
SAD	2.2 ± 0.4	7.7 ± 1.9*	11.8 ± 2.5*	2.6 ± 0.7	9.0 ± 1.5*	11.5 ± 2.7*
MFC	2.0 ± 0.5	4.7 ± 0.5* #	4.3 ± 0.9* #	5.2 ± 1.3	8.3 ± 1.6	7.6 ± 1.2
SAD+MFC	3.1 ± 0.8	7.7 ± 0.6*	9.6 ± 1.5*	6.3 ± 1.2	13.6 ± 3.6*	14.3 ± 3.8*
			CSr RESPONSES			
Control	1.5 ± 0.2	3.0 ± 0.5* #	2.8 ± 0.6 #	5.1 ± 1.0	7.1 ± 1.3	7.5 ± 1.6
SAD	2.7 ± 0.9	7.2 ± 1.4*	4.9 ± 1.0*	3.9 ± 0.7	5.5 ± 1.0	6.6 ± 2.2
MFC	2.5 ± 0.5	5.4 ± 0.5*	2.9 ± 0.4 #	4.9 ± 1.5	7.1 ± 1.2	5.4 ± 0.7 #
SAD+MFC	3.2 ± 1.1	7.9 ± 1.3*	6.7 ± 0.7*	7.1 ± 2.9	11.9 ± 3.2	8.1 ± 1.0

CS+ heart rate and blood pressure variability ± 1 SEM. Baseline is defined as the mean of seconds 9 to 19; CS is defined as the mean of seconds 21-31; Late is defined as the time remaining in the trial (seconds 40 through 108). * denotes responses significantly different from baseline; # denotes responses significantly different between corresponding baroreceptor-intact group.

Figure 21. Neuroanatomical representation of possible connections involved in the regulation of cardiovascular CS+ responses in baroreceptor-intact Controls and SAD lesioned rats. i, infralimbic region of MFC; p, prelimbic region of MFC; A/H, amygdala and hypothalamus; NTS, solitary nucleus; IML, intermediolateral cell column; BA, baroreceptor afferents. ⟶⟶⟶ denotes long latency direct activation of parasympathetic component by A/H; - - - denotes inhibition of normal response by prelimbic MFC; + denotes excitation. A. Control CS+ response. Open arrow represents increased HR in response to stress. B. SAD lesion CS+ response. * denotes denervation of baroreceptor afferents; filled arrow represents increased HR response (compared to Controls) in response to stress following SAD.

A



B

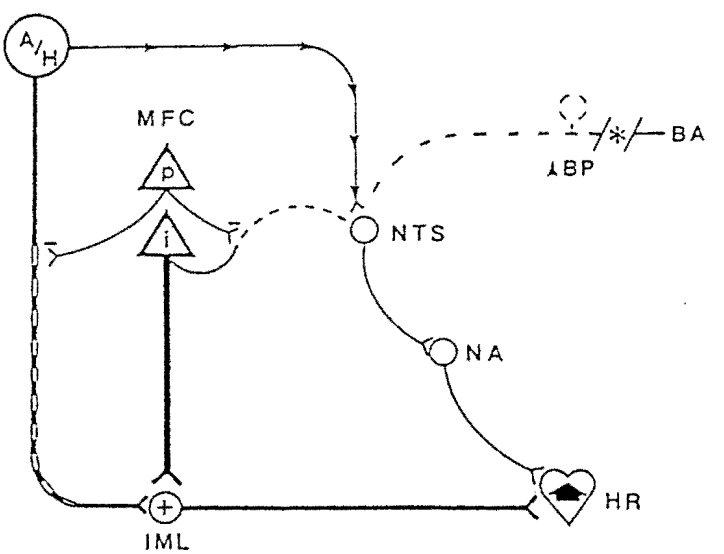
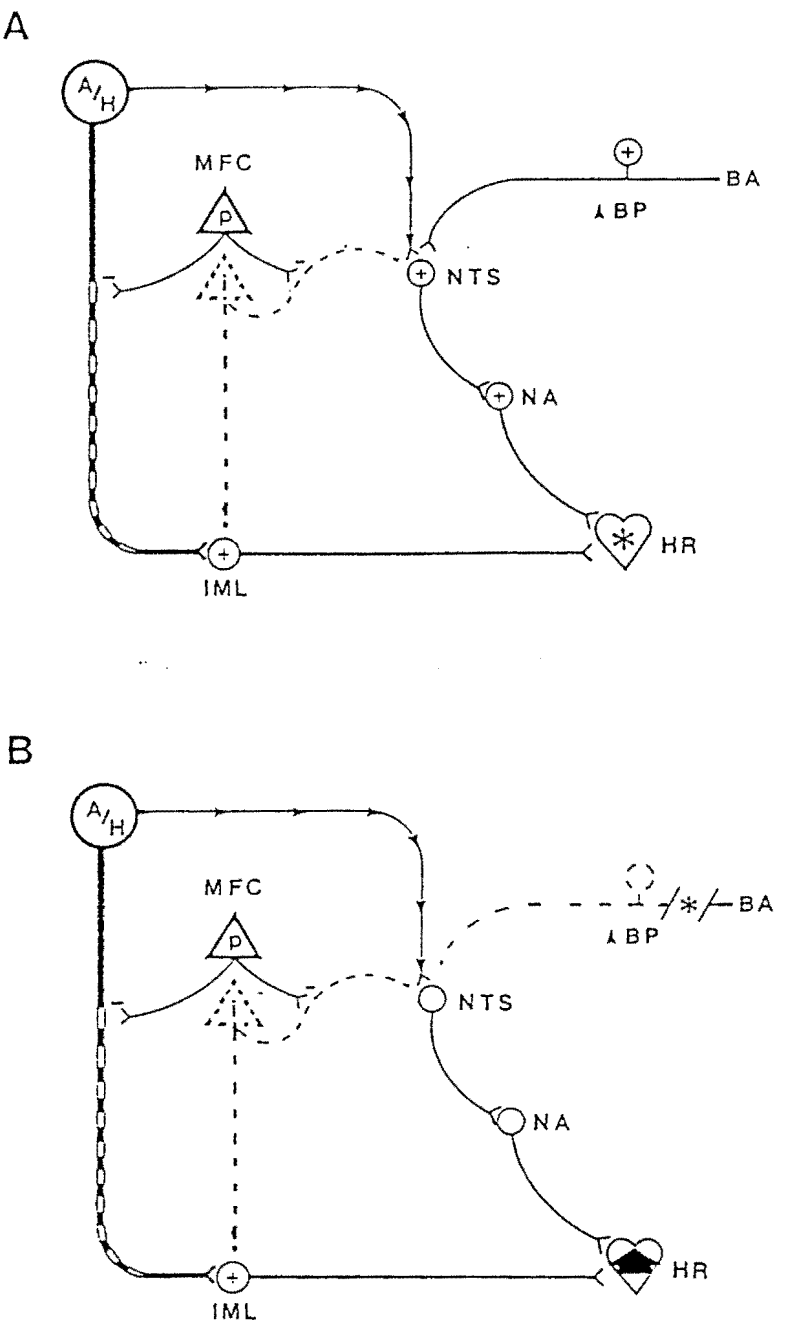


Figure 22. Neuroanatomical representation of possible connections involved in the regulation of cardiovascular CS+ responses in baroreceptor-intact MFC and SAD+MFC lesioned rats. i, infralimbic region of MFC (dotted lines indicate lesion of this area); p, prelimbic region of MFC; A/H, amygdala and hypothalamus; NTS, solitary nucleus; IML, intermediolateral cell column; BA, baroreceptor afferents. ⟶⟶⟶ denotes long latency direct activation of parasympathetic component by A/H; - - - denotes inhibition of normal response by prelimbic MFC; + denotes excitation. A. MFC lesion CS+ response. * represents slow-onset bradycardia in response to stress. B. SAD+MFC lesion CS+ response. * denotes denervation of baroreceptor afferents; filled arrow represents tachycardia in response to stress following SAD+MFC lesion, which was significantly different from MFC lesion response.


A
A/H
MFC
P
BA
ΔBP
NTS
NA
HR
IML
B
A/H
MFC
P
BA
ΔBP
NTS
NA
HR
IML

CHAPTER VIII

RESPIRATION, VOCALIZATION, AND FREEZING RESPONSES

INTRODUCTION

Previous behavioral studies of cardiovascular responses to stress in rats have ignored respiration (Grossman, 1983), even though respiration is known to be a powerful modulator of cardiovascular activity via both the vagus (Katona et al., 1970) and sympathetic nervous system (Cohen & Gootman, 1970; Barman & Gebber, 1976). The anticipation of electric shock during a perceptual task leads to a significant reduction in end-tidal $C0_2$ levels and increases in respiratory rate and thoracic movement (Suess et al., 1980). Rapid breathing is associated with the psychological characteristics of anxiety, depression, phobic behavior, and high levels of perceived and objective stressors. Cardiovascularly, reduced parasympathetic tone and increased sympathetic dominance are expressed by increased heart rate and cardiac output, reduced RSA and baroreceptor responsiveness, and increased incidence of ECG abnormalities (Cedres et al., 1982; Eckberg, 1980; Grossman, 1983). A dramatic series of changes in respiration occur when a rat is placed in a naturally stressful situation, such as defeat (Fokkema et al., 1986). Initially the rat pants at a very rapid rate (approximately 5 Hz, much faster than the resting rate of 1.5 Hz) for 15 to 20 seconds, followed abruptly by a period of slow, deep breathing (approximately 0.8 Hz) that lasts several minutes and is often accompanied by ultrasonic vocalizations. Vagal activation is associated with slow, deep breathing, and vagal withdrawl increases with faster, shallower breathing, during which time sympathetic responses can become dominant (Grossman, 1983). This latter phase is also characterized by immobility (freezing), elevated blood pressure, pronounced

fluctuations of blood pressure corresponding to inspiration, and greatly increased heart rate variability, which appears to be a respiratory sinus arrythmia (RSA). These observations establish respiration and associated behavioral changes, such as ultrasonic vocalizations and freezing, as critical variables that must be included in any study of stress-related cardiovascular changes.

The specific aims of this portion of the study were to determine the normal resting pattern of respiration and RSA in rats; to determine the normal respiratory, freezing, ultrasonic vocalization and RSA responses during the CS+ and CSr responses; and to determine what effects MFC lesions, SAD lesions, and SAD + MFC lesions had upon these responses.

The procedures used during classical conditioning, cannula implantation, recording of data, animal sacrifice and data analysis are found in the "General Methods" chapter (4) of this dissertation. Procedures for NMDA MFC lesions can be found in the methods section of chapter 5; procedures for SAD lesions can be found in chapter 7.

Respiration

Respiration was monitored by measuring the transthoracic impedance between the ECG chest electrodes with a UFI model 2991 impedance converter. Output of the device was sent continuously to the polygraph, and sampled by the computer at the occurrence of the R-wave in the ECG signal (approximately 5 Hz). This signal was converted to mean respiratory rate (breaths per minute) over 2 second sampling periods. To permit across animal averaging, responses were determined as changes from baseline. Within group comparisons were made by multiple comparisons (Bechhofer-Dunnet) to determine responses significantly different from baseline. Between groups comparisons at corresponding timepoints were made by ANOVA followed by t-tests.

Ultrasonic Vocalizations and Freezing

Ultrasonic vocalizations were monitored on an oscilloscope via a high frequency condenser microphone (Panasonic PF9932). The signal was also input

directly into the amplifier of the polygraph; the driver output of the pen (which integrated the signal; see chapter 4) was then sampled by the computer at each R wave. Latency to the onset of vocalizations and the duration of vocalizations were determined from the original polygraph recordings, since many of the rats continued to vocalize after the end of the computer sampling window (120 seconds). Freezing was also noted on and measured from the polygraph tracings.

Respiratory Sinus Arrhythmia Analysis

RSA was assessed during a 2 minute baseline trial (no CS) and during the early and late phases of the CER. The "peak-trough" method (Schectman et al., 1987) was used to measure heart rate period RSA. The signal to be analyzed (heart period) was subjected to an iterative procedure, which on each pass through the data defined either peaks (x-1 <x> x+1) or troughs (x-1 >x< x+1). The time between adjacent peaks was defined as the "period" of the peak; the peak's amplitude was equal to the height (in msec) of a vertical line extending from the subsequent trough to its intersection with an interpolated line between the peak and the subsequent peak. Variation in a given frequency range was quantified by the median extent of all period values from that pass. At the end of the first pass through the data, a set of data pairs was collected: periods (in msec) between peaks and the amplitude (in msec) of the peaks (see Figure 23). The median values obtained from the first pass generally fell within the respiratory frequency range of 0.5 to 5.0 Hz. If it did not, then a second pass, using the "peaks" identified in the

first pass, was performed. Such an analysis was performed on the baseline trial and during three epochs of the CER trial for each animal in Groups 1 and 2. Within group comparisons of the amount of RSA during different epochs was made using analysis of variance and Dunn's test applied to the means of the median values for each period (amplitude and period). Between groups comparisons of the amount of RSA during comparable epochs was made using the Bechofer-Dunnett multiple comparison procedure for independent samples, again analyzing the means of the median values.

The chronological order of the procedures each rat was subjected to during this portion of the study were:

1. SAD lesion/sham - 2 week recovery period
2. MFC lesion/sham - 2 week recovery period
3. Classical conditioning of animal, followed by implantation of cardiovascular and respiratory recording hardware - 24 hour recovery
4. Recording of cardiovascular, respiratory, and ultrasonic vocalization CERs
5. Recording of cardiovascular, respiratory, and ultrasonic vocalization responses during US presentation
6. Sacrifice by anesthetic overdose
7. Data analysis

Comparison of Resting Values

The normal resting respiratory rate was calculated for each group from the two minute baseline trial prior to CER testing, during which no tones (CS+ or CSr) were given. No significant differences were found between any of the groups, indicating that neither MFC or SAD lesions had any significant effects on resting respiratory rate. Normal grooming, nesting, and feeding behaviors were observed both during and after the CER trials in all animals. Spontaneous ultrasonic vocalizations were not emitted by any of the rats prior to testing; SAD lesioned rats could not vocalize due to the loss of laryngeal innervation.

Respiration Responses

Figure 24 illustrates the normal pattern of blood pressure, heart rate, ultrasonic vocalization and respiration during typical CS+ and CSr trials for Control and MFC lesioned rats. Although the chart speed is too slow to discern individual breaths, note that the respiratory pattern seen in Controls following the CS+ (A) is distinctly different from both its baseline and the corresponding CSr response (B). This difference can be attributed to the onset of USV, which is lacking following the CSr. Additionally, note that the respiratory pattern in the MFC lesioned responses (C & D) does not display any obvious differences between the CS+ and CSr responses, except for those attributed to movement artifacts, and that there is no evidence of USV.

Figure 25 illustrates both the average respiratory responses for each group (Figures A & B) as well as the mean baseline, CS, and Post CS responses (Figures C & D) during the CS+ and CSr presentations. Respiratory rate increased significantly during the CS+ in all groups when compared to baseline. The mean increase for MFC, SAD and SAD+MFC groups was significantly larger than the Control CS+ response (Figure 25C). Following the CS+, the mean respiratory rate for Control and SAD lesioned rats decreased significantly below baseline, while MFC and SAD+MFC lesioned rats remained significantly above baseline. These differences in respiratory rate following the CS+ were also significant between MFC-intact groups (Control and SAD) and MFC lesioned groups (MFC and SAD+MFC). The decrease in respiratory rate seen in Controls was due to the onset of vocalizations. Forced expirations, which mimic the pattern of respiration seen in vocalizing Controls, were also noted in SAD lesioned rats, indicating that they were probably attempting to vocalize (but were unable due to denervation of the larynx). Neither MFC nor SAD+MFC rats produced or attempted to produce USV.

During the CSr, respiratory rate increased significantly compared to baseline for all groups, then quickly returned to baseline (Figure 25B). The CSr response in SAD lesioned rats showed a smaller increase than Controls (Figure 25D). Respiratory rate was not significantly different from baseline throughout the remainder of the trial for all groups except MFC lesioned rats, which remained significantly elevated.

USV and Freezing Responses

Freezing began at the onset of the CS+ for all animals studied and persisted past the end of the recording trial (120 seconds) for Control and SAD lesioned rats (Figure 26A). MFC and SAD+MFC lesioned rats displayed significantly shorter freezing times than Controls or SAD rats following the CS+. During CSr trials, freezing was significantly reduced for all groups compared to CS+ responses, with many animals failing to show any freezing behavior.

Ultrasonic vocalizations were seen in 92% of Controls following the CS+, while only 18 % of MFC lesioned rats emitted USV (Figure 26B). The onset of USV coincided with a significant increase in cardiovascular variability (see Figure 24A), and also had a clear effect on the respiratory rate and pattern. Figure 27A displays a typical Post CS+ response. The rats often vocalized in small bursts of USV, during which the rat took a quick inspiration, followed by a prolonged expiration associated with vocalization. Between these bursts of USV, the rats panted at approximately 4 Hz. Heart rate and blood pressure oscillations were correlated with this respiratory pattern, such that during inspiration, blood pressure and heart rate decreased, while during forced expiration (USV), they increased. The frequency of the ultrasonic vocalizations was determined via direct waveform inspection of the USV signal of all animals (Figure 27B). Additionally, spectral analysis was performed in two animals (Figure 27C-D). The frequency varied from animal to animal and from trial to trial within animals, but the overall average frequency of vocalization was 26.4 $\pm$ 4.1 kHz (SEM), with a mean duration of 862

± 54.3 msec (SEM). During a single vocalization, the frequency was relatively constant.

The mean duration of vocalizations for those MFC lesioned rats which did vocalize was significantly shorter than Controls (Table 12). A brief delay was noted between the offset of the tone and the beginning of USV; vocalizations did not occur unless the rat was in the crouched "freezing" posture. Following CSr presentations, only 23% of Controls emitted USV, and the latency and duration of these vocalizations was significantly shorter than the CS+ responses (Figure 26B; Table 12). The animals which did vocalize following the CSr were those which had shown prolonged freezing and USV following the CS+. Only one MFC lesioned rat emitted USV following the CSr, and the duration of USV was very brief.

Unconditioned Stimulus (US) Responses

Following all trials, the rats were placed back into the plexiglass shock chamber where acquisition occurred. The animals each received one US (2 mAmp footshock, 0.5 sec duration). In all cases, the US response consisted of a large increase in blood pressure paired with a strong tachycardia. Ultrasonic vocalizations began immediately following the US and persisted vigorously through the end of the trial, lasting up to 10 minutes in some cases. Respiration assumed the pattern seen during all other USV responses (see Figure 26A), namely quick inspirations followed by prolonged, forced expirations. The patterns of these responses in rats with MFC lesions were not different from those seen in Controls

or SAD lesioned rats. The tachycardia and occurrence of USV during the US trial was significantly different, however, for the MFC lesioned group when compared to the MFC CS+ response, which consisted of a slow-onset bradycardia and no USV.

Heart Period RSA Analysis

Resting RSA values computed at four timepoints during the 2 minute baseline trial showed no significant deviations in amplitude or period from computed baseline values either within or between any of the groups studied. Significant differences were detected, however, during the CER.

Analysis of the HR intervals during the CS+ (Figure 28) showed significant decreases in period for all groups compared to baseline, corresponding to increased respiratory rate during the CS+; the apparent decrease in amplitude was not significant. RSA period during the Post CS2 phase (seconds 80-90) was significantly increased in Control and SAD lesioned groups compared to baseline. This increase in period corresponded to the mean decrease seen in respiratory rate during the late CS+ phase, as well as the increased variability in the HR signal mentioned earlier. This increased variability and the overall increase in RSA HR period correlate with USV, which was not seen in MFC lesioned rats. RSA amplitude was not significantly altered from baseline during the late phase. The lack of a significant increase in HR period during the late phase in MFC and SAD+MFC lesioned rats corresponds to the lack of variability seen in the cardiovascular signals (see Figure 24), which was due presumably to the conspicuous lack of USV.

DISCUSSION

Rats with an intact MFC (Control & SAD lesioned rats) responded initially to the CS+ with increased respiratory rate and freezing behavior, which was subsequently followed by decreased respiratory rate, prolonged freezing, and USV (due to the loss of the superior laryngeal nerves, SAD rats did not display USV). This resembles the pattern previously described by Fokkema et al. (1986) during social stress following defeat in rats. Fokkema attributed the synchronous oscillations in BP and respiration to a "Valsalva-like" maneuver: the rat took a quick, deep inspiration followed by a prolonged, forced expiration. During inspiration, BP and HR decreased, while during the forced expiration BP and HR increased. This was the pattern we observed during the late "USV" phase of the CS+ in Controls (Figure 27A). In our study, USV were always associated with this pattern of respiration, occurring in bursts separated by brief periods of panting. Analysis of the frequency of the USV revealed that the rats were vocalizing in the 22 to 30 kHz frequency range. Similar vocalizations have been found in subordinate male rats during agonistic behavior (Sales, 1972; Thames et al., 1983), following footshock (Tonoue et al., 1986), following exposure to attacking opponents (Berg & Baeninger, 1973), after daily handling (Sales, 1979), and during the post-ejaculatory refractory state in males (Barfield & Geyer, 1975). It has been proposed that USV in the 22 to 30 kHz band indicates that the rat producing the call was in a withdrawn emotional state (Anisko et al., 1978) and may be related to the amount of fear the animal was experiencing (Tonoue et al., 1986).

Conversely, those animals in which the MFC was removed (MFC and SAD+MFC lesioned rats) responded to the CS+ with sustained increases in respiratory rate, reduced freezing, and virtually no USV. Anatomically, the MFC of the rat projects heavily to the ipsilateral periaqueductal gray (PAG)(Terreberry & Neafsey, 1987; van der Kooy et al, 1982, 1984; Neafsey et al., 1986). The PAG in the rat projects to laryngeal motoneurons in the nucleus ambiguus (Bystrzycka & Nail, 1985), and has been shown to have a significant influence on vocalization in the rat (Yajima & Hayashi, 1983 a & b), the cat (Holstege, 1989; Kanai & Wang, 1962) and the monkey (Larson & Kistler, 1984, 1985). The PAG has been implicated in fear responses (Irisawa & Iwasaki, 1982; Wada et al., 1970), rage (Bandler, 1982), and other defensive and emotional behaviors (DiScala et al., 1987). PAG stimulation has been used as an aversive US (Irisawa & Iwasaki, 1982; DiScala et al., 1987), and vocalizations have been produced by stimulation of this area in many species, including the rat, cat and monkey (Jürgens & Pratt, 1979; Kanai & Wang, 1962; Larson & Kistler, 1984, 1985). Lesions of the PAG result in the loss of vocalizations (Adametz & O'Leary, 1959; Jürgens & Pratt, 1979), while recording of neuronal activity in the PAG has been related to vocalization and laryngeal EMG activity (Larson & Kistler, 1984, 1986; Larson et al., 1988). Lesions of the MFC, therefore, would eliminate an input to the PAG, potentially affecting vocalization output.

The primate homologue of the rat MFC, the anterior cingulate cortex, has also been implicated in vocalization in monkeys (Smith, 1945; Robinson, 1967;

Jürgens & Ploog, 1970; Müller-Preuss & Jürgens, 1976; Sutton et al., 1981; Kirzinger & Jürgens, 1982) and man (von Cramon & Jürgens, 1983). Lesions of the midline frontolimbic cortex that completely remove areas 24 and 25 in the squirrel monkey result in loss of vocalizations (Aitken, 1981; Newman, 1988; MacLean & Newman, 1988); incomplete lesions of these frontolimbic areas does not result in this loss. MacLean & Newman postulate that anatomical connections from the frontal cortex to the PAG and from the PAG to the nucleus ambiguus suggest that the medial frontal cortex may be responsible for the expression of vocalizations.

Heart period RSA analysis displayed significant decreases between baseline and CS+ responses in all groups, corresponding to the increased respiratory rate during the CS+. RSA is known to be an accurate indicator of vagal cardiac control, since a high correlation exists between RSA and parasympathetic tone (Akselrod et al., 1981; Grossman, 1983). Parasympathetic outflow during the CS+ would therefore appear to be diminished, due to the significant decreases seen in RSA period analysis. The results from Chapter 5 indicated that parasympathetic tone was initially low during the CS+, displaying a slow-onset activation as time progressed.

Following the CS+, respiration oscillated between very slow periods (during USV) and very fast periods (during panting between USV bursts)(see Figure 27A). Calculation of RSA from these values introduced a high degree of variability into the results, making significant comparisons between visible differences extremely difficult. A similar finding was related by Grossman & Svebak (1987), who stated

that "quantification techniques ... may misestimate RSA when respiration rate is either very slow or very fast." During the late phase of the CER, period measurements were significantly different between MFC-intact (Control & SAD lesioned) and MFC lesioned groups (MFC & MFC+SAD). These differences correspond to the differences seen in respiration (MFC-intact decreased; MFC lesioned increased) and vocalizations (MFC-intact vocalized; MFC lesioned did not vocalize) between these groups.

The responses associated with the US responses indicate that the "learned" response to the CS+ and CSr were mediated by brain areas other than, or in addition to the brain areas mediating the US response. During the US, it is likely that pain pathways and other sensory input contributed to the profound pressor response, tachycardia and vocalizations. Lesions of the MFC did not prevent vocalizations during the US, indicating that the MFC is not essential for production of USV. This supports the hierarchy proposed by Ploog (1981), who categorized the structures related to the output of vocalizations into 4 categories. The highest (primary) level of vocal organization was considered to be the anterior limbic cortex, which projects directly to both secondary motivational areas (central nucleus of the amygdala, lateral hypothalamus, mediodorsal nucleus of the thalamus) and the tertiary integration area (PAG: Ploog, 1981). The anterior limbic cortex is thought to induce calling in conditioned situations (Ploog, 1981), and may be responsible for voluntary use of voice in man (Ploog, 1981; Ploog & Jürgens, 1980). Both the primary and secondary levels of vocal control project directly to the PAG

(tertiary level), which integrates the vocal cues and then projects directly to the output pathway of the nucleus ambiguus. The pattern of USV following the US was identical in both MFC and Controls, and was also identical to the CS+ mediated USV seen in vocalizing Controls. These results indicate that the MFC does not directly affect the pattern of vocalization, which appears to be controlled by the PAG (Ploog, 1981; Holstege, 1989), but does affect the motivational or voluntary output pathway processed by the secondary vocalization structures.

CONCLUSIONS

All animals responded to the CS+ with increased respiratory rate and freezing, and a significant decrease in RSA period; no animals vocalized during the actual CS+ presentation.

Following the CS+, RSA period was significantly increased in Controls and SAD lesioned animals compared to baseline. Controls and SAD lesioned rats displayed prolonged freezing, decreased respiratory rate, and USV (Controls only). These behaviors indicate a sustained fear response following the actual CS+ tone. MFC and MFC+SAD lesioned animals displayed significantly decreased periods of freezing compared to Controls, and virtually no USV; respiratory rate slowly returned to baseline by the end of the trial. These behaviors indicate a significantly reduced stress response in MFC lesioned animals compared to Controls, implying that the MFC may be necessary for behavioral responses such as freezing, and motivational responses such as vocalization.

Figure 23. RSA peak-trough analysis of heart period. Peaks (open triangles) and troughs (t), and their associated amplitude (A_1 - A_4) and period (P_1 - P_4) values are shown on a portion of original heart rate data. Analysis of the *period* values determined that the average heart rate period over 10 seconds was 752 $\pm$ 103 msec, or 1.33 HZ, which is within the respiratory frequency band (0.5 to 5.0 Hz).

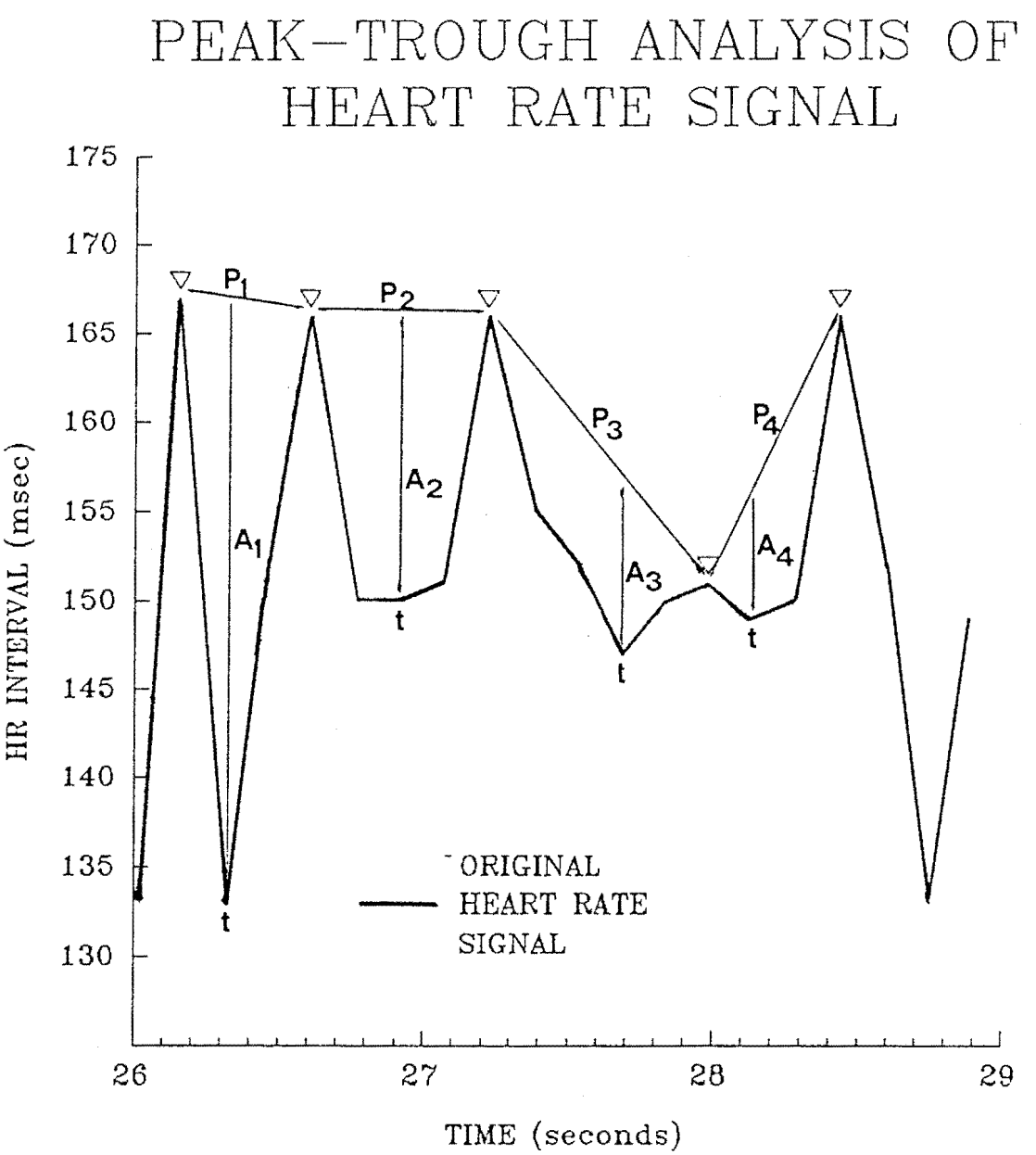

PEAK–TROUGH ANALYSIS OF HEART RATE SIGNAL
175
170
165
160
155
150
145
140
135
130
HR INTERVAL (msec)
P1
P2
P3
P4
A1
A2
A3
A4
t
t
t
t
ORIGINAL HEART RATE SIGNAL
26
27
28
29
TIME (seconds)

Figure 24. Polygraph tracings of individual Control and MFC lesioned CS+ and CSr responses. Blood pressure (BP), heart rate (HR), ultrasonic vocalizations (USV) and respiration (RESP) tracings; bar represents occurrence of tone. A. Control responses during the CS+ presentation. B. Control responses during the CSr presentation. C. MFC lesioned group responses during the CS+ presentation. D. MFC lesioned group responses during the CSr presentation. Oscillations appearing late in respiration (*) were due to movement artifacts.

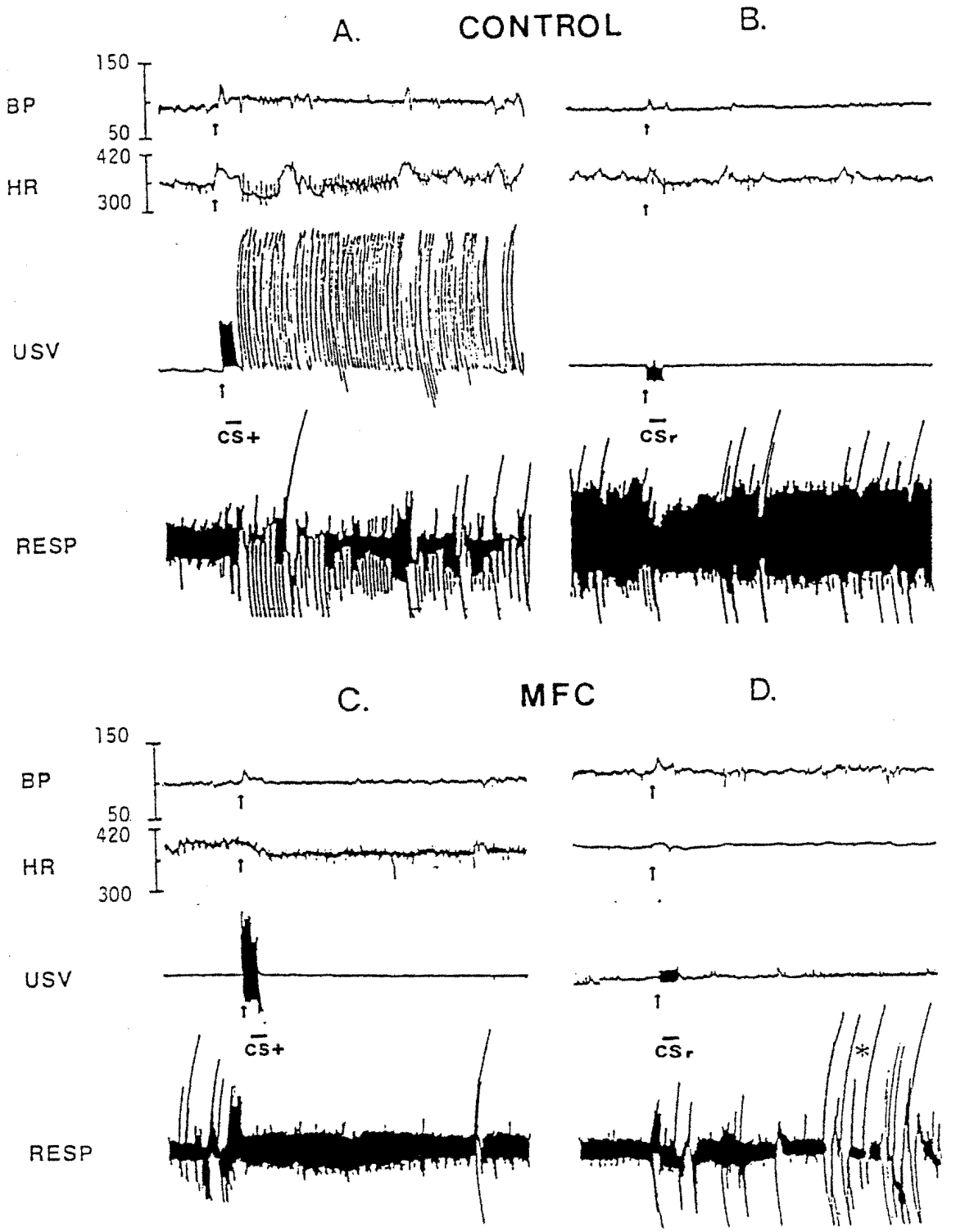

A.
CONTROL
B.
150
50
BP
420
300
HR
USV
CS+
CS-
RESP
C.
MFC
D.
150
50
BP
420
300
HR
USV
CS+
CS-
RESP
*

Figure 25. CS+ and CSr respiratory responses. A & B. Average respiratory responses plotted as changes from baseline (breaths per minute) vs time. Solid lines denote Control responses; Dotted lines denote MFC responses; Dot-Dot-Dash lines denote SAD lesioned responses; Dot-Dash lines denote SAD + MFC lesioned responses. A. CS+ responses. B. CSr responses. C & D. Bargraphs showing mean respiratory rate $\pm$ 1 SEM during BASELINE, CS and POST CS portions of the CER. Responses are shown as mean rate in breaths per minute for each group. Open bars denote Control; filled bars denote MFC; right-hatched bars denote SAD; left-hatched bars denote SAD+MFC. * indicates responses significantly different from Control response; ▼ indicates responses significantly different from Control in corresponding time period. C. CS+ responses. D. CSr responses.

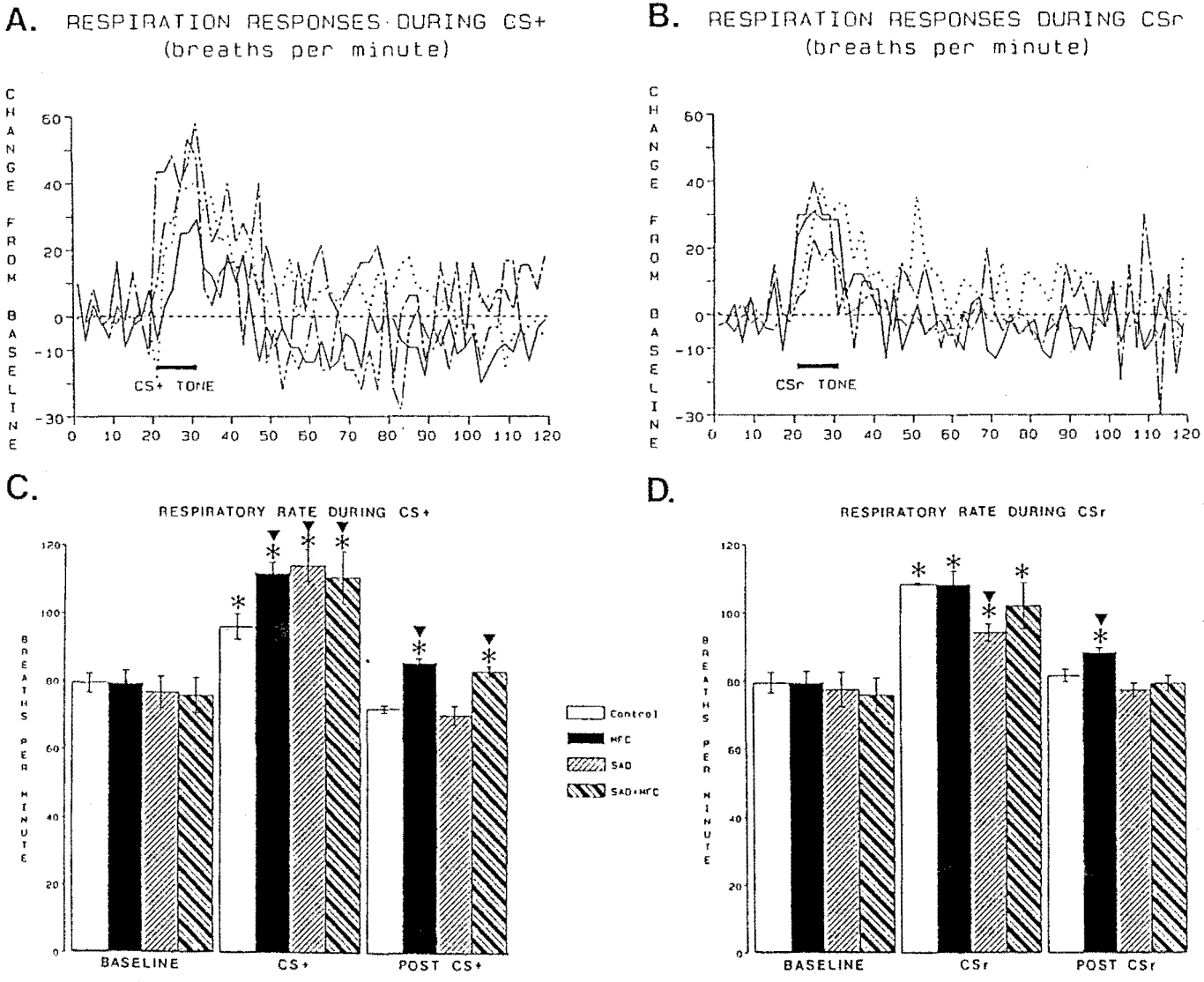
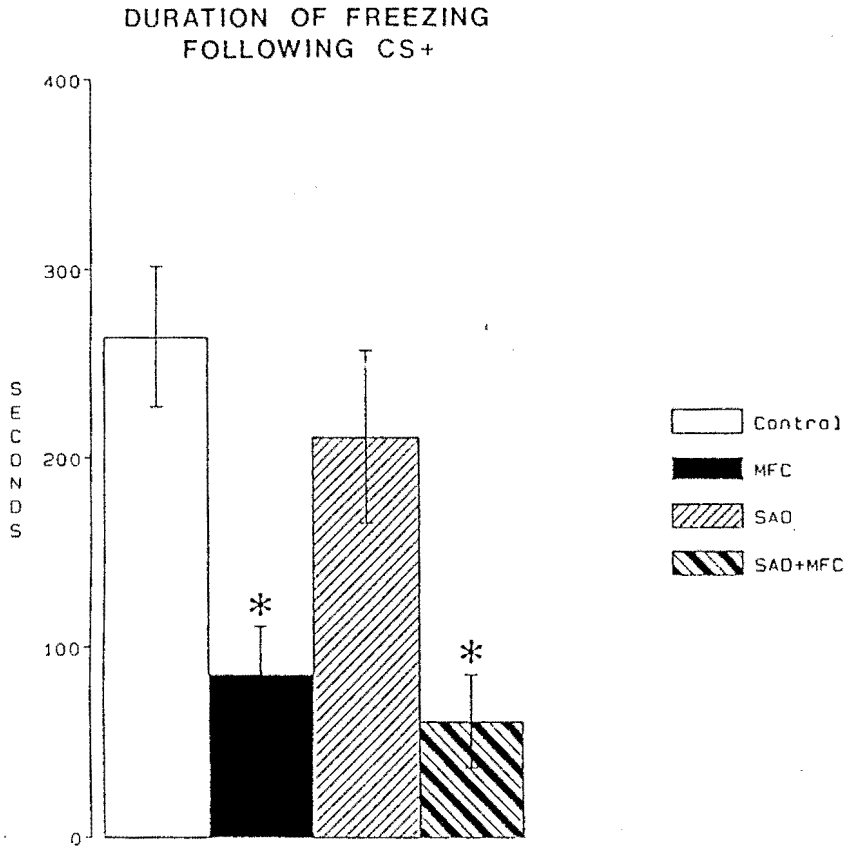

A. RESPIRATION RESPONSES DURING CS+
(breaths per minute)
CHANGE FROM BASELINE
CS+ TONE
B. RESPIRATION RESPONSES DURING CSr
(breaths per minute)
CSr TONE
C.
RESPIRATORY RATE DURING CS+
BREATHS PER MINUTE
BASELINE
CS+
POST CS+
Control
MFC
SAD
SAD+MFC
D.
RESPIRATORY RATE DURING CSr
BREATHS PER MINUTE
BASELINE
CSr
POST CSr

Figure 26. Behavioral responses during the CER. Open bars denote Control rats; filled bars denote MFC lesioned rats; right-hatched bars denote SAD lesioned rats; left-hatched bars denote SAD+MFC lesioned rats. A. Bargraph depicting the duration of freezing during the CS+ trial. Responses plotted as mean duration of freezing in seconds $\pm$ 1 SEM. * denotes responses significantly different from Control response. B. Bargraph depicting percent of animals vocalizing during either CS+ or CSr trials.

A.


DURATION OF FREEZING
FOLLOWING CS+
SECONDS
400
300
200
100
0
*
*
Control
MFC
SAD
SAD+MFC

B.

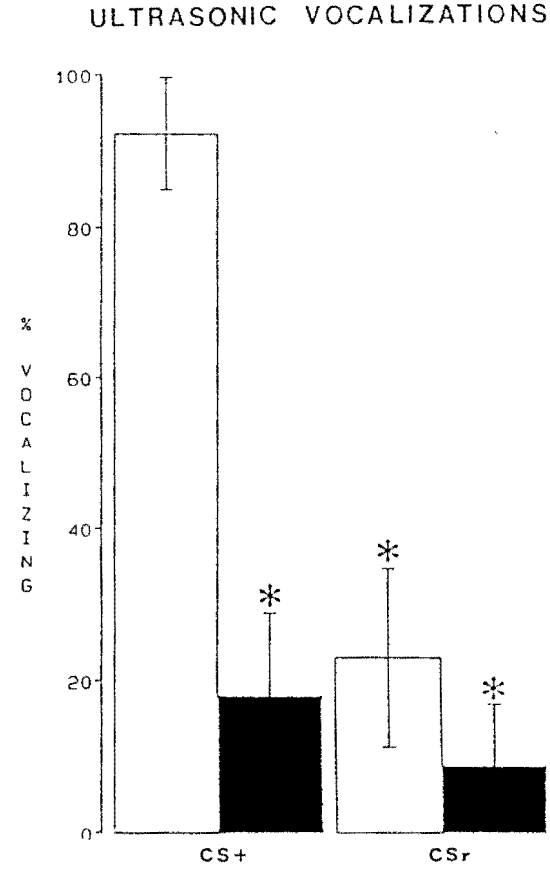

ULTRASONIC VOCALIZATIONS
% VOCALIZING
100
80
60
40
20
0
*
*
CS+
CSr

Table 12. **CER VOCALIZATION RESPONSES**

SUBJECT (N)	LATENCY	LENGTH OF USV
	CS+ RESPONSES	
Control (13)	27.2 ± 11.1	208.3 ± 41.4
MFC (11)	36.4 ± 17.3	18.2 ± 6.8*
	CSr RESPONSES	
Control (13)	8.7 ± 1.8	22.3 ± 6.4
MFC (11)	9.0 (N = 1)	9.0 (N = 1)

Behavioral analysis of USV for CS+ and CSr responses, expressed in seconds ± 1 SEM. (SAD and SAD+MFC lesioned rats were unable to vocalize, and therefore USV responses were not studied) * denotes responses significantly different from Control response ($p<0.05$). Latency refers to the time delay between the offset of the tone and the onset of USV.

Figure 27. Vocalization analysis. A. Oscilloscope tracing displaying blood pressure, heart rate, USV, and respiration; bar = 2 seconds; inspiration is down. Note decrease in BP and HR during inspiration, and increase in BP and HR during expiration; USV coincide with expiration. Note also that between USV's (arrow), the rat pants at approximately 4 Hz. B. Oscilloscope tracing of direct waveform analysis of the USV signal following a CS+ tone. 1) Microphone signal at slow sweep speed, displaying a typical "burst" of USV. Bar = 2 **seconds.**
2) Microphone signal at high-speed trace displaying waveform of a portion of a typical USV. Arrows denote "peaks" in waveform used to calculate the frequency of the USV. Bar = 0.2 **milliseconds.** C & D illustrate oscilloscope tracings of the frequency spectral analysis during the late phase of the CS+ response in a Control animal during vocalizations. C illustrates microphone signal recorded during an 800 msec bin before the cursor was placed over the peak response (arrow). D illustrates the same tracing seen in C after the cursor has marked the frequency of vocalizations (arrow) = 24.875 kHz. Note that the frequency of USV is relatively constant.

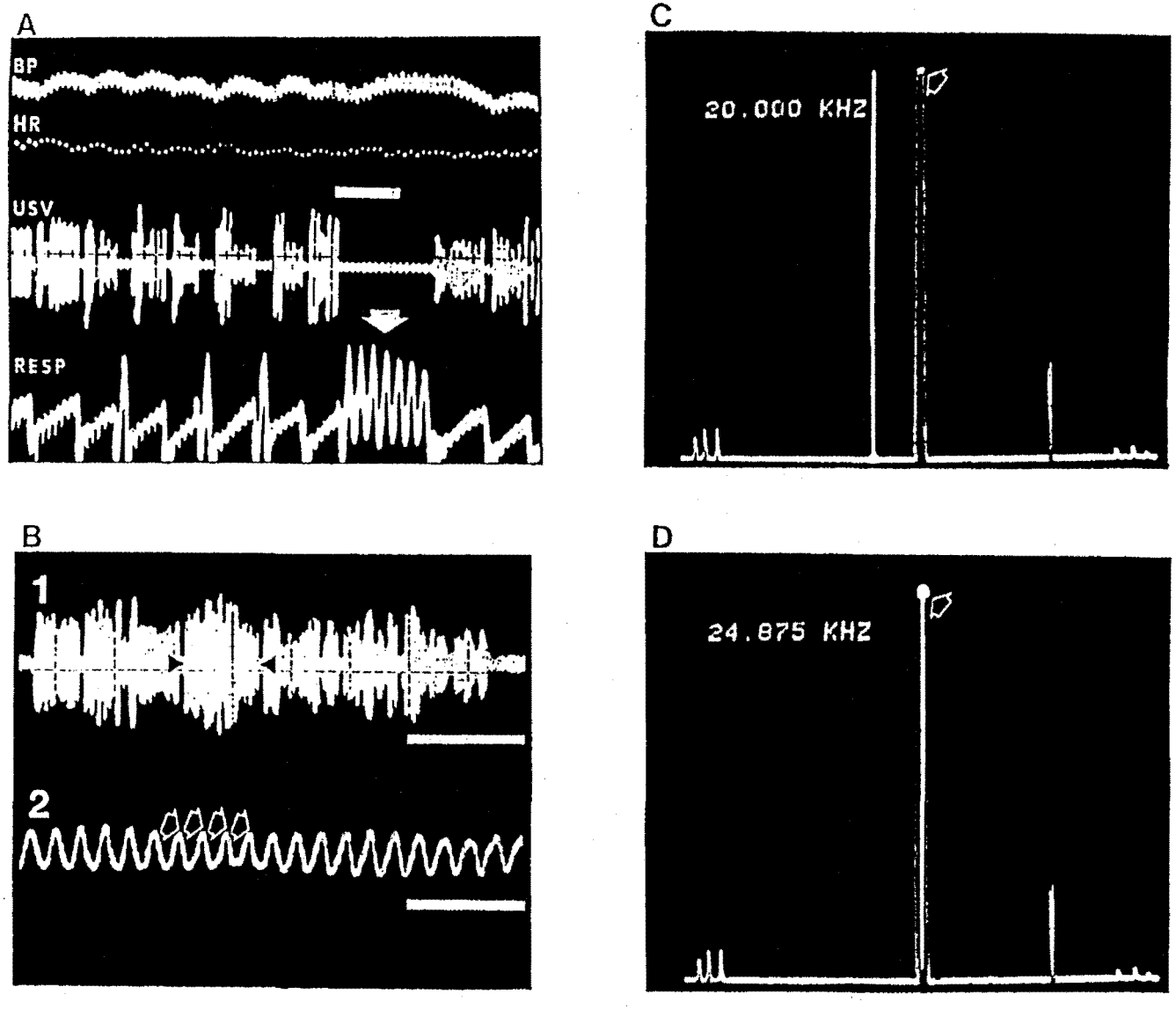
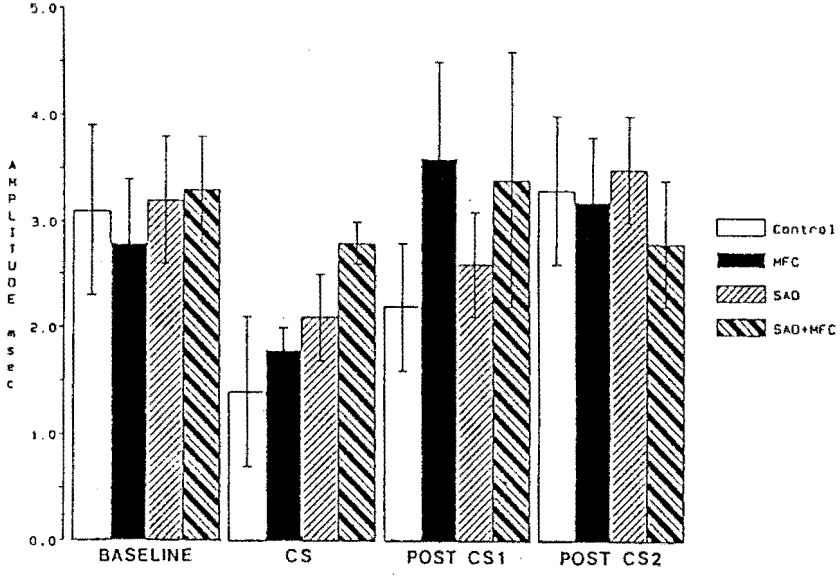
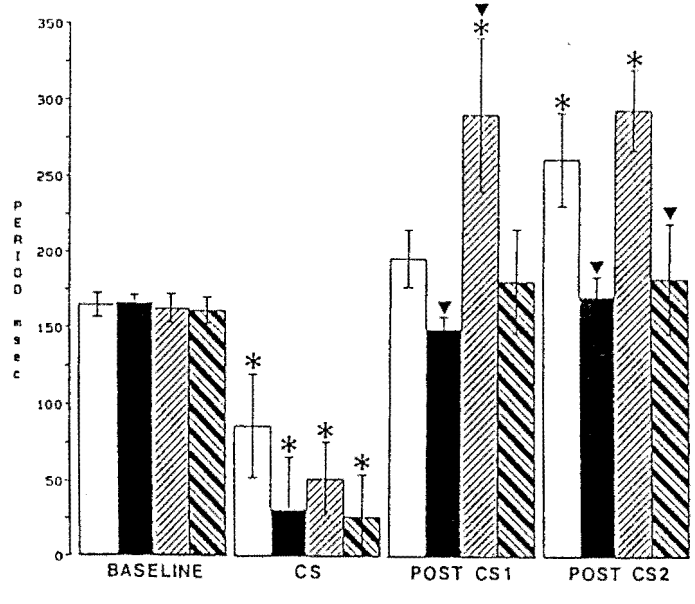

A
BP
HR
USV
RESP
B
1
2
C
20.000 KHZ
D
24.875 KHZ

Figure 28. RSA amplitude and period analysis. Open bars denote Control group; filled bars denote MFC lesioned group; right-hatched bars denote SAD group; left-hatched bars denote SAD+MFC lesioned group. * denote responses significantly different from baseline; ▼ denotes responses significantly different from Control response. All responses plotted as mean response in msec $\pm$ 1 SEM. Baseline was determined by the mean response during seconds 9-19; CS+ response was determined by the mean response during seconds 21-31; Post CS1 was determined by the mean response during seconds 32-42; Post CS2 was determined by the mean response during seconds 80-90. A. CS+ amplitude responses. B. CS+ period responses.

A.


RSA AMPLITUDE ANALYSIS
AMPLITUDE msec
5.0
4.0
3.0
2.0
1.0
0.0
BASELINE
CS
POST CS1
POST CS2
Control
MFC
SAD
SAD+MFC

B.


RSA PERIOD ANALYSIS
PERIOD msec
350
300
250
200
150
100
50
0
BASELINE
CS
POST CS1
POST CS2

CHAPTER IX

GENERAL DISCUSSION

AND SUMMARY

GENERAL DISCUSSION

Despite the tremendous difference in the relative size of the frontal cortex in mammals, as well as the obvious diversity of behavioral and social patterns, there appears to be a remarkable consistency in the functions of the frontal cortex across mammalian species (Kolb, 1984). Many studies in man, monkey and rat have explored the behavioral, autonomic and motor deficits related to frontal cortex lesions. Table 13 highlights some of the more prominent behavioral and autonomic functions attributed to the frontal cortex in rats, as determined by lesion studies. Many of the same or similar functions, such as temporal ordering, response inhibition, spatial orientation, social or affective behaviors, behavioral spontaneity and habituation have also been studied in man following frontal lobe lesions (see review by Kolb, 1984). The deficits in man observed include perseverative errors in card-sorting tasks (Milner, 1964; Konorski, 1964), poor maze learning (Corkin, 1965), impaired social behavior (Blumer & Benson, 1975), reduced verbal fluency and spontaneous talking (Milner, 1964; Kolb & Taylor, 1981), reduced facial expression (Kolb & Milner, 1981), impaired habituation of orienting reactions (Luria & Homskaya, 1964), and aphasia (Brown, 1972; Damasio, 1981, 1985). According to Fuster (1980), the principal function of the frontal cortex is to provide a temporal framework for behavior, organizing various behaviors and responses into a meaningful whole. The results of this dissertation support this view and extend it to include both autonomic and somatic responses to emotional stress.

One of the first to attempt to "localize" emotion in specific brain areas was Papez (1937), who proposed that a circuit involving the hippocampus, hypothalamus, anterior thalamic nuclei, cingulate gyrus, and hippocampus constituted a "harmonious mechanism" for experiencing and expressing emotion. Papez viewed the cingulate gyrus as the receptive area for experiencing emotion, thereby activating the hippocampus and neocortex. MacLean (1949) expanded Papez's circuit to include the amygdala, septal nuclei, olfactory areas, basal ganglia, and insular and frontal cortices; these structures he termed the limbic system. While Papez's original hypothesis concerning his "circuit" has proven to be incorrect or unsubstantiated, MacLean's original concept that the components of the limbic system are involved in emotion and visceral function has persisted (see review by Issacson, 1982).

LeDoux et al. (1988) stated that the different projections of the central nucleus of the amygdala to the central gray and lateral hypothalamus independently mediate the behavioral and autonomic correlates of conditioned fear. Lesions of the caudal central gray result in decreased freezing behavior, while lesions of the lateral hypothalamus reduced the conditioned mean arterial pressure responses. Lesions of the central nucleus of the amygdala resulted in reduction of both behavioral (freezing) and autonomic (cardiovascular) responses (Kapp et al., 1979; Gentile et al., 1986; Hitchcock & Davis, 1986; Iwata et al., 1986). These results have clearly established the amygdala and associated regions as important elements in the brain's "emotional circuit."

The amygdala, however, is not the only brain region which controls such behaviors. The prefrontal cortex has been shown to have specific increases in dopamine turnover in the rat during stress (Thierry et al., 1976). Following medial frontal lesions in the rat, the gain of the cardiac baroreflex is known to be reduced (Verberne et al., 1986), and increased "timidity" has been noted (Holson, 1986; Holson & Walker, 1986). Lesions of the subcallosal anterior cingulate region of the frontal cortex in monkeys (areas 24, 25) abolish or alter vocalizations and/or distress calls (Sutton et al., 1974; MacLean & Newman, 1988; Aitken, 1981). These observations, combined with the data presented here, indicate that specific regions of the frontal cortex can be associated with autonomic and "emotional" functions.

Nauta (1971) proposed that lesions of the frontal cortex result in an "interoceptive agnosia," characterized by an absence of normal visceral responses. For example, when a person is about to perform in front of a large audience for the first time, he experiences the well-known phenomenon of "butterflies in the stomach." According to Nauta, the frontal cortex plays a large role in producing this visceral response. Without the frontal cortex, this "gut feeling" would be absent or significantly reduced, and the person would be ignorant (agnosia) of what his body would normally be telling him. This may also help explain the often inappropriate behavior seen in people with frontal lobe lesions.

The normal response in a rat to a conditioned stimulus previously paired with footshock is to freeze and "cry out" (vocalize); the autonomic response resembles a cardiovascular defense response (increased BP and HR). If the MFC is

responsible for producing the visceral and behavioral responses to such stimuli, then lesions of the MFC would alter or eliminate these responses, leading to interoceptive agnosia or visceral ignorance. The CS may still be perceived correctly as the precursor to the US, but the autonomic and behavioral responses to the CS are no longer produced correctly. The result is a significant decrease in the expression of behaviors associated with stress. The rat does not vocalize; freezing is dramatically shortened; autonomic responses are no longer driven by the sympathetic nervous system, and therefore the heart rate response is dramatically altered when compared to the normal CS response. The MFC, therefore, is an essential component for the integration and subsequent expression of behavioral and autonomic responses to stress. This finding is consistent with both MacLean's (1949) original description of the limbic system as the "visceral brain," and the conclusions reached by Nauta in 1971. Nauta concluded that telencephalic limbic structures were intimately involved in controlling an animal's internal milieu, and that their functional states were manifested via affects and motivation. If this cortical area could be controlled, either pharmacologically or consciously, many of the symptoms associated with stress, such as cardiovascular disease and stroke, might be dramatically reduced.

SUMMARY

The physiological and behavioral changes associated with lesions of the infralimbic portion of the rat medial frontal cortex during the conditioned emotional response have been investigated. The normal (Control) CS+ CER consisted of an initial increase in blood pressure, heart rate and respiration, along with a reduction of the cardiac baroreflex gain during the CS+ tone. These early responses were followed by a prolonged period of increased cardiovascular variability, freezing, ultrasonic vocalizations, and slow, "pressure" breathing. The cardiovascular responses were mediated by coactivation of the sympathetic and parasympathetic nervous systems, with the sympathetic component being dominant. Medial frontal cortex lesions resulted in a significant reduction in the normal CS+ tachycardia response, while the late response showed significant decreases in freezing, USV, and cardiovascular variability compared to Controls. The cardiac baroreflex gain was permanently reduced after MFC lesions, and no gain changes occurred during the CER. The CS+ cardiovascular responses were mediated by coactivation of the sympathetic and parasympathetic nervous systems, but the sympathetic component was significantly reduced (-52%), resulting in the parasympathetic component being dominant.

The Control CSr CER showed significantly smaller cardiovascular responses and no changes in baroreflex gain during the CSr tone presentation; late responses showed little freezing or USV activity, and increased cardiovascular variability and

pressure breathing were not observed. The CSr response was mediated only by activation of the sympathetic nervous system. The MFC lesioned CSr response consisted of very small increases in blood pressure and heart rate, and no freezing or USV were detected. The MFC CSr response was mediated only sympathetic activation, but at a significantly reduced level compared to Control CSr responses.

Table 13. **FUNCTIONS OF THE MEDIAL FRONTAL CORTEX**

Behavior/Response	Nature of Response Following MFC Lesion	Reference
Response Inhibition	Impaired performance on tasks requiring changing behavior	Kolb et al., 1974
Spatial Orientation	Poor spatial learning	Kolb, 1983
Social Behavior	Increased fighting; homosexual behavior	Lubar, 1973; Kolb, 1974c
Habituation	Head poke prevented/retarded;	Glaser, 1962; Butter, 1964; Kolb, 1984
Contralateral Neglect	No response to sensory stimuli	Crown, 1982
Food Hoarding	Significantly decreased	Stamm, 1954; Kolb, 1974b; Whishaw, 1989
Maternal Behaviors	Poor pup retrieval; Nest building impaired	Stamm, 1955; Wilsoncroft, 1963; Shipley,1977
General Activity	Typically increased	Richter, 1939; Kolb, 1974a
Cardiovascular CER	Baroreflex reduced; Hypertension reversed	Verberne, 1987; Szilagyi, 1987; present study
Vocalizations	Number and length of USV significantly reduced	present study
Freezing Behavior	Freezing time significantly shortened	present study

CHAPTER X

LITERATURE CITED

LITERATURE CITED

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The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Anatomy.

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