THE EFFECTS OF AMYGDALAR LESIONS ON HEART RATE RESPONSES IN RATS

by

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The present study was designed to determine if lesions in the amygdala affected the nature of conditioned and unconditioned heart rate responses using a classical conditioning paradigm. There were no significant differences between the heart rate responses to the CS+ and CS- during the conditioning period in either the lesioned or sham-operated group. In addition there was no evidence of an orienting response in the heart rate to the initial presentations of either tone in either of the two groups.

These findings led to further investigation of the practice of breaking the tympanic membrane to insure correct placement of a rat in the stereotaxic apparatus. The results of a bar-press study suggest that this procedure results in serious impairment of the animal's hearing, which is not regained in a 2-week recovery period.

It appeared that the lesioned animals were more responsive to the shock than the sham-operated ones. This result is consistent with the hypothesis that the amygdala has a role in modulating emotional responding to noxious stimuli.

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INTRODUCTION

Pavlovian conditioning of heart rate has been thoroughly documented. Sherrington (1900) noted disturbances in the heart rate of dogs in response to a rattle, which had regularly preceded shock. Gantt (1960) performed numerous experiments on conditioned heart rate in dogs, verifying and elaborating this finding. However, progress in defining the role of the central nervous system in the development of the heart rate conditioned response has been confined to more recent history.

Aversive Classical Conditioning of Heart Rate

Studies have been performed concerning classically conditioned heart rate changes in several species (e.g. Cohen & Durkovic, 1966; Fitzgerald & Teyler, 1970; Obrist, 1968; Powell, Schneiderman, Elster, & Jacobson, 1971). This research has defined the parameters involved in establishing the conditioned response. In addition there have been several attempts to conceptualize the pertinent factors of cardiovascular plasticity into a theoretical framework.

The stimulus-substitution theory, which originated with Pavlov, states that pairing a conditioned stimulus (CS) and an unconditioned stimulus (US) leads to the formation of an association between them such that the CS alone comes to elicit the set of responses normally elicited by the US. Although much evidence can be marshalled in defense of this theory (Mackintosh, 1974), heart rate conditioning presents

certain difficulties for the theory which limit its usefulness in this situation. For example, in heart rate conditioning of restrained rats a deceleratory CR develops to a tone CS when paired with a shock US that often produces an acceleratory UR (e.g. Fitzgerald & Teyler, 1970). In addition, when the direction of the CR change is consistent with the direction of the UR, the topography of the response may differ (Sideroff, Schneiderman, & Powell, 1971). Given that the US determines the form or direction of the CR, then the CRs for any given US should be the same. On the contrary, Powell et al (1971) found that changing the sensory modality of the CS without changing the US could alter the direction of the CR.

Some motivational theories also stress the relationship between the CR and US. Sideroff, Elster, and Schneiderman (1972) demonstrated that the form and the direction of the heart rate CR was the same for both appetitive and aversive USs, indicating that this dichotomy does not satisfactorily explain variations in CRs.

Obrist (1976) has proposed that heart rate and skeletal motor activity are interrelated and that this relationship is governed by common processes in the central nervous system. According to this view, the direction and amplitude of heart rate CRs may depend on the somatic response the training procedure demands. However, there has been no neural organization described to date that would provide a

mechanism for such an interaction. On the contrary, Martin and Fitzgerald (1980) found significant correlation between heart rate and skeletal motor responses in restrained rats, but not in unrestrained rats. In both groups, heart rate changes were noted in the absence of any change in movement. These data suggest that heart rate changes may be augmented by appropriate changes in movement, but are not dependent on them.

Some theorists have emphasized the role of the CR as part of an attentional process (e.g. Lacey & Lacey, 1970; Sokolov, 1963). Sokolov has related the elicitation of autonomic CRs to neural mechanisms that modulate the reception of sensory input. More precisely, low-to-moderate intensities of stimulation elicit autonomic and somatic responses (i.e., the orienting reflex) that facilitate afferent input. Very intense stimulation, on the other hand, elicits a different constellation of responses (i.e., the defensive reaction) that protects the organism from overstimulation by inhibiting sensory input. It is not known whether CRs differentially influence sensory input, but the direction of the heart rate CR does not appear to be directly related to attentional processes as described by Sokolov.

Schneiderman (1974) has speculated that the CR is an adaptive response which prepares the organism to either augment or cope with the effects of the US, depending on their implications for the organism. If the pattern of

responding is adaptive to the organism, the CR serves to facilitate it; if it is disturbing or maladaptive, there will be a compensatory change. For example, Schneiderman (1974) showed that the URs to an intracranial injection of acetylcholine included an increase in heart rate and a decrease in blood pressure. The cardioaccelerative CR which developed could be considered as a preparatory response, which helped to compensate for the decrease in blood pressure elicited by the US. The thought was that the CR would prevent blood pooling in the veins. This theory does not provide any obvious explanation for why restrained rats can have a decelerative heart rate CR with an accelerative heart rate UR.

The "sensitization hypothesis" suggests that the heart rate conditioned response may be generated from or share common elements with the cardiac component of the orienting response (Fitzgerald, 1976). Early studies by Fitzgerald and others (Fitzgerald & Teyler, 1970; Fitzgerald & Martin, 1971) were consistent with a sensitization view of the conditioning process. Although the conditioning process produced some changes in the CR which distinquished it from the orienting response, certain components of the orienting response were still present.

Later work has not supported the position that the heart rate CR develops directly out of the heart rate orienting response. Fitzgerald and Hoffman (1976a) demonstrated that habituation, which one would expect to extinquish the

orienting response, tends to enhance the heart rate conditioning response. Fitzgerald, Stainbrook, Francisco, and Hoffman (in preparation) used a low intensity shock CS paired with a high intensity shock US. The original accelerative heart rate orienting response to the CS did not lead to accelerative CRs; the dominant CR that appeared during conditioning was decelerative. These studies suggest a relationship between the CR and the orienting response, but the nature of it is unclear.

In restrained rats, the heart rate CR to a tone is deceleratory when paired with a shock US, even when the UR is acceleratory. On the other hand, it is possible to alter the direction of the heart rate response in restrained rats by changing the temporal relationship between the CS and US (Cunningham, Fitzgerald, & Francisco, 1977). Cardio-accelerations developed as CRs to a CS- in animals that received an explicitly unpaired regimen with a CS- and a US (previously paired with a CS+). Based on the results of combined-cue and conditioning tests, this explicitly unpaired procedure appeared to produce conditioned inhibition. These data open up the possibility that the direction of cardiac activity may reflect the behavioral state produced by the contingency between CS and US.

Due to the complex nature of the interrelationships involved in cardiovascular conditioning, it is not surprising that none of these conceptual frameworks can account for the

data available or even a major part of them. But the explanations do offer insight into the variables that may be relevant in an explanation of neural control of the conditioned resonse.

Neural Control of the Heart Rate Conditioned Response

Behaviorally, the experimental model has been well documented. The next step in determining the role of the central nervous system in the development of the heart rate CR is the specification of neuroanatomical pathways necessary for the normal development of the learned response. One important aspect of this process and the one with which this study is concerned, is mapping the pathway(s) for the motor outflow which produces the CR. The most systematic efforts to achieve this goal have attempted to trace the pathway(s) rostrally from the nerves that innervate the heart.

Analysis of the Final Common Pathway of Motor Output

Before analyzing the nerve pathways, a preliminary question of primary importance is whether or not the conditioned response is mediated by sources other than extrinsic cardiac nerves. Cohen and Pitts (1968) demonstrated that no conditioned heart rate responses developed in pigeons with bilateral vagotomy and beta adrenergic blockade. McDonald and Cohen (1970) also showed using surgical methods that no conditioned heart rate responses could be established in pigeons that had complete cardiac denervation. These studies indicate that in the time period observed (a 6-8 sec CS-US

interval), hormonal or indirect neural influences do not produce an observable CR.

Another factor to be considered is the relative contribution of vagi and cardiac sympathetics to the conditioned heart rate response. For the design used in this experiment (tone CS paired with shock US in restrained rats), the decelerative heart rate CR is controlled primarily by increased vagal output with relatively minor involvement of the sympathetic system. Fitzgerald, Martin, and O'Brien (1973) demonstrated that when vagal input to the heart was blocked with atropine, there was no observable heart rate conditioned response. Testing during extinction without atropine showed a CR had developed. Similar results have been found in the rabbit (Schneiderman, VanDercar, Yehler, Manning, Golden, and Schneiderman, 1969). These results indicate that preparations which show conditioned decelerations do so primarily by increasing vagal output.

The first step in the anatomical tracing of pathways mediating the motor outflow involved in producing the CR is to localize the medullary origins of the vagal cardio-inhibitory fibers. Nosaka, Yamamoto, and Yasunaga (1979) mapped an extensive area of the brainstem in an effort to locate areas that elicited bradycardia with a low threshold stimulus and yielded a large bradycardia response to a given suprathreshold stimulation. Then they compared the cell populations which met these criteria with cell populations

labelled with HRP injected into the cranial cardiac branch of the vagus nerve. Taken together the results of these studies indicated that the vagal cardioinhibitory pre-ganglionic neurons in the rat are located in the dorsal motor nucleus of the vagus, the region surrounding the nucleus ambiguus, and the intermediary zone lying between the two. These results concur with those of Schwaber and Schneiderman (1975) who showed that the dorsal motor nucleus of the vagus contains some cells which were vagal cardioinhibitory neurons in the rabbit. The cells they studied responded antidromically to stimulation of the cervical vagus and orthodromically to stimulation of the aortic nerve (baro-receptor afferents).

The Role of the Amygdala in Cardiovascular Function

Up to this point the number of alternative structures that could be involved in the neural pathway sought here was minimal. But then it was necessary to choose from several more rostral brain structures that had been implicated in the control of the cardiovascular system. Much of the information available was based on electrical stimulation and therefore only provided an approximation of the neural circuitry involved. Considering the present interest in finding the pathway that mediates the conditioned response to an aversive stimulus, it was logical to choose a structure capable of modulating cardiovascular responding to stressful conditions, rather than one involved in the

maintenance of normal resting states.

Ethologists have used the term agonistic behavior to describe the various kinds of adaptation that occur during conflict or threat. Two of these types of emotional responses have been produced by stimulation of the amygdala in the cat. They are the flight reaction and the defense reaction. In the flight response the animal first appears restless and looks in all directions; it then withdraws or escapes without growling or hissing. There are signs ofsympathetic outbursts with pupillary dilatation and sometimes piloerection. In the defense reaction the animal initially exhibits the orienting behavior described above. It then retracts its head and crouches. The ears are flattened to a posterior position, the animal growls and hisses, the pupils are dilated, and there is piloerection. On stronger stimulation the animal may raise a forepaw, ready to strike with protruded claws (Kaada, 1972). Hilton and Zbrozyna (1963) have shown that electrical stimulation in the amygdala of the cat can produce the defense response which includes profound changes in heart rate, blood pressure, and regional blood flow. The responsive region was located in the medial portion of the basal nucleus, overlapping to include parts of the lateral portion of basal nucleus and the central nucleus. Flight behavior has resulted from stimulation in an area extending from the rostral part of the lateral nucleus and the peri-amygdaloid area through the region of the central nucleus

and into the ventral part of the internal capsule (Kaada, 1972).

Amygdalar stimulation produces predominantly pressor responses in unanesthetized cats (Reis & Oliphant, 1964). The strongest effects have been elicited from the areas previously associated with flight and defense behavior, including an area corresponding to the course of the ventral amygdalofugal pathway (Kaada, 1972). A map published by Wood, Schottelius, Frost, and Baldwin (1958) shows components of flight and defense reactions in the basal and central nuclei of the rat. Faiers, Calaresu, and Mogenson (1975) demonstrated that arterial hypotension could be elicited at low current intensities from the medial, central, lateral, and basal nuclei of rats under urethan or chloralose.

Reis and Oliphant (1964) found responsive loci for bradycardia in all subdivisions of the amygdala in cats, but particularly in the basomedial part. Tachycardia followed stimulation of points which were clustered around areas from which bradycardia was elicited. The tachycardia points were concentrated in the white matter of the external and internal capsule, but tachycardia could be elicited from all amygdaloid subdivisions and surrounding paleocortex. Bonvallet and Gary Bobo (1972) studied the effects of localized repetitive stimulation of the amygdala on heart rate in cats under gallamine triethiodide (Flaxedil). They frequently found cardiodeceleration when stimulating the

lateral part of the central nucleus, the lateral nucleus, the parvocellular part of the basal nucleus, the peri-amygdaloid cortex, and the putamen. They obtained cardio-acceleration in more restricted areas, including the magnocellular part of the basal nucleus. Stimulation to this area resulted in immediate cardiodeceleration, followed by a delayed cardioacceleration. Mogenson and Calaresu (1973) found that stimulation of the medial, basolateral, and central nuclei of the amygdala produced bradycardia (18-20 beats per minute) in most instances, but the effect did not approach statistical significance.

There is an obvious lack of consistency across these studies, but a number of explanations for this problem exist (e.g., anesthetic level, sensory input, species differences, etc.). Or if one conceptualizes the amygdaloid structures as playing a modulatory role, then one might assume that the response observed would be based on the state of the animal, as well as on the particular locus of stimulation.

In summary, the cardiovascular system (particularly heart rate and blood pressure) is responsive to stimulation in the amygdala and surrounding areas. In some cases the response patterns are strikingly similar to what one sees in animals reacting to situations involving conflict or threat.

Afferent and Efferent Connections of the Amygdala

Recently developed neuroanatomical techniques (e.g.,

HRP and autoradiography) have made it possible to begin tracing central autonomic neurons, whose highly branched axonal systems with little or no myelination proved troublesome for techniques used previously. Hopkins and Holstege (1978) reported that ^{3}H leucine injections into the amygdala of cats produced transported label concentrated within the rostrocaudal vagal nuclei (i.e., the dorsal motor nucleus of the vagus and the nucleus tractus solitarius). The nucleus tractus solitarius is an area of primary vagal and glossopharyngeal baroreceptor afferent termination (Jordon & Spyer, 1977). Schwaber, Kapp, and Higgins (1980) did a follow-up study in the rabbit to examine the extent of neurons in the amygdala labeled by retrograde axonal transport of HRP injected into the rostrocaudal vagal nuclei. They found that HRP-labelled cells were confined to well-defined regions of the amygdala and surrounding basal forebrain structures. The total field of labeled cells appeared unbroken, forming a continuum extending in a dorsomedial and rostral direction from the central nucleus through the sublenticular substantia innominata beneath the globus pallidus and internal capsule and into the lateral part of the bed nucleus of the stria terminalis.

These results are consistent with findings that the central nucleus is also primarily the origin of recently reported amygdalar projections to other lower brainstem structures, such as the parabrachial nucleus of the pons,

which may play a role in autonomic regulation (Hopkins & Holstege, 1978; Krettek & Price, 1978). The area of projection found by Schwaber et al (1980) is a prominent target for projections from the basolateral nucleus of the amygdala (Krettek & Price, 1978). Ricardo and Koh (1978) demonstrated that this continuum also receives projections from the commissural part of the nucleus of the solitary tract. So this area appears to both directly project to and receive input from primary visceral nuclei. Taken together these results document a direct projection from the forebrain to the dorsomedial medulla, which may be part of the anatomical substrate for the forebrain control of the cardiovascular aspects of the emotional behavior described earlier.

Amygdalar Involvement in the Acquisition of Heart Rate Conditioned Responses

There have also been a few studies which have indicated that the amygdalar complex plays a role in the normal acquisition of the heart rate CR. Cohen (1975) found that destruction of the posterio-mediale region of the archistriatum or interruption of its descending projection produced profound deficits in the development of the accelerative CR in visual classical conditioning of heart rate in the pigeon. Based on an anatomical analysis Zeier and Karten (1971) have suggested that the posterior-mediale archistriatum constitutes the avian amygdalar homologue. Plunkett (1978) found that in the rat amygdalectomy resulted in cardio-

deceleration to a light which had been paired with shock that did not occur in unoperated controls. Paired presentation of tone and shock did not produce different response patterns to the tone in the two groups. However, in neither case was there a control to distinquish learning from sensitization. Kapp, Frysinger, Gallagher, and Haselton (1979) showed that rabbits receiving either a small or large lesion of the central nucleus of the amygdala failed to achieve the same magnitude of conditioned bradycardia to a tone CS paired with a shock US as was found in sham-operated controls.

The Role of the Amygdala in Arousal, Agonistic, and Avoidance Behavior

The potential for a significant role of the amygdala in the development of conditioned heart rate responses is not surprising considering the relationship of this complex to other brain areas and the number of learned and unlearned behaviors to which it has been linked. Anatomically it is part of the limbic system and the differentiation of the various nuclei within the amygdala has remained essentially unchanged from marsupials to man. The study of more primitive animals reveals a phylogenetic division into a more primitive centromedial group and a more recent basolateral group (including the lateral and basal nuclei) (Johnston, 1923). The centromedial group is composed of central, medial, and cortical nuclei and the nucleus of the lateral olfactory tract. More recently Koikegami has proposed a different

subdivision based on fiber projections and functional studies (Kaada, 1972). The important difference from the earlier one is that the small-celled part of the basal nucleus is included in the centromedial group. Study of the afferent connections to the amygdala is becoming more and more refined (Ottersen, 1979, 1980). Of particular interest are the connections from other limbic structures, hypothalamic nuclei, cortical areas, and the brain stem. Knowledge of efferent connections is also expanding (Krettek & Price, 1977, 1978). Monosynaptic connections include various hypothalamic nuclei, basal ganglia, as well as numerous connections within the limbic system.

The role of the amygdala in other behaviors may provide insights into its function in aversive classical conditioning of heart rate. Three behaviors, which have been studied extensively in this way are a) general arousal and orienting, b) agonistic behavior (including flight, defense, and predatory attack), and c) avoidance learning (active and passive).

General Arousal

Following bilateral removal of the amygdala, animals are frequently lethargic for a week or more, but this gradually declines and the animal may become hyperactive. This hyperactivity appears to be based on an increased reactiveness to environmental stimuli, particularly visual stimuli (Goddard, 1964). The orienting response is the

most common response elicited by amygdaloid stimulation. It is quite similar to the orienting response induced by stimulation of the reticular formation, except that it habituates more rapidly (Ursin & Kaada, 1960a; Ursin, Wester, & Ursin, 1967). Arousal, associated with searching or orienting movements, can be elicited from the anterior amygdaloid area, the lateral and magnocellular parts of the basolateral nucleus, and the area extending medially through the region of the central nucleus and into the internal capsule just dorsal to the optic tract in the region of the entopeduncular nucleus (Ursin & Kaada, 1960b). Cortical desynchronization was obtained from essentially the same amygdaloid areas as the orienting response (Kreindler & Steriade, 1964).

The amygdala appears to be essential for some components of the orienting response in monkeys. Bilateral removal of this structure causes a depression of the GSR, heart rate, and respiratory rate components of the orienting response, while the EEG activation and ear movement components remained essentially intact, but failed to habituate (Bagshaw & Benzies, 1968). On the other hand there has been no significant effect on heart rate orienting response in rats with amygdalar lesions (Holdstock, 1969) or in rabbits with central amygdalar lesions (Kapp et al, 1979). In conclusion there is some evidence that the amygdala is involved in the control of arousal and orienting, but the specific nature

of this control remains unclear.

Agonistic Behavior

Emotionality and responsiveness to noxious stimuli are the most controversial and frequently studied phenomena to be linked with amygdaloid function. In most studies bilateral removal of the amygdala results in placidity (e.g., Rosvold, Mirsky, & Pribram, 1954). This lack of emotion involves a loss of aggression, a reduced responsiveness to normally noxious stimuli, and a completely fearless curiosity for dangerous or threatening stimuli including members of other species. Rage is not eliminated, but has a very high threshold. The placidity is relatively permanent and control lesions indicate that it is primarily due to the removal of the amygdala (Kaada, 1972). However, a few studies have reported rage (e.g. Wood, 1958).

Miczek, Brykczynski, and Grossman (1974) did a systematic follow-up study to compare the effects of lesions in the periamygdaloid cortex, in specific amygdalar nuclei, or in the bed nucleus of the stria terminalis on intraspecies and interspecies aggressive behaviors in the male rat. They found that small lesions in the periamygdaloid cortex, cortical amygdaloid nucleus, or the bed nucleus of the stria terminalis reduced or eliminated attacks and signs of dominance in fights, which were generated by isolated housing and the omission of food rewards. They also found that small lesions in the lateral or central amygdaloid

nuclei significantly inhibited the "aggressive" reactions to footshock (rearing and boxing), which they feel might be most productively viewed as "defensive" behaviors. They conclude that there may be distinct neural pathways for the two response types.

The role of the amygdala in fear and aggression has also been studied using electrical stimulation. Flight and defense zones have been elicited from separate, but parallel zones, as defined earlier. In addition the amygdala has been shown to play a role in the regulation of the endocrine system, especially during stress (e.g. Knigge, 1961; Mason, 1959).

Avoidance Behavior

It has been proposed that the amygdala is primarily involved in the regulation of fear. A large number of studies have addressed this issue using active and passive avoidance paradigms. In the majority of early studies, the lesions involved all of the amygdala or extensive parts of it. After reviewing this literature, Goddard (1964) concluded that the lesions in the amygdala disrupt avoidance learning more than approach learning, providing some support for the fear hypothesis. Blanchard and Blanchard (1972) demonstrated that large lesions of the amygdaloid area in rats showed reduced freezing to an immobile cat or to a previously neutral stimulus that had been paired with shock. Based on these results they postulate that the amygdala is involved

in the regulation of an emotional-motivational state necessary for the elicitation or maintenance of a defense reaction, rather than the formation of an association between a neutral stimulus and a painful one when the two are paired.

More recently studies have attempted to localize this effect to particular amygdalar areas. One would predict that lesions in areas previously shown to be involved in facilitating flight would reduce the ability to exhibit escape responses and to learn active avoidance behavior. By contrast lesions in areas involved in inhibition of responding would result in decrements in passive avoidance learning (basolateral nucleus of the amygdala) (Kaada, 1972). Ursin (1965) found that lesions restricted to the flight zone in the rostral part of the lateral nucleus or damage to the ventral amygdalofugal pathway in cats resulted in impaired active avoidance behavior, whereas passive avoidance was not influenced by these lesions. Horvath (1963) reported similar findings with amygdaloid lesions which appear to involve the flight zone. He found a learning deficit only for the two-way active avoidance procedure, but not for the one-way active or passive avoidance procedures. One could argue that the two-way avoidance procedure is more dependent on fear motivation and would be more seriously impaired by removal of a fear-producing neural locus. Pellegrino (1968) showed that rats with bilateral lesions

of the basolateral region of the amygdala were impaired in passive avoidance, which supports Kaada's premise that the basolateral region is concerned with inhibition of responding (1972).

Werka, Skar,and Ursin (1978) examined the effects of central, basolateral, and cortical lesions on several behavioral tasks. Lesions in the central nucleus produced increased activity in an open-field test, but the other groups were unchanged. In one-way active avoidance all groups with lesions showed deficits, but the most pronounced were in the central group. In an earlier study by Coover, Ursin, and Levine (1973), rats with amygdalar lesions showed lower plasma levels of corticosterone during the acquisition of a two-way avoidance task, indicating less "fear" than controls. Werka et al (1978) concluded that taken together these data indicate that lesions of the central nucleus may act to reduce fear. Further support for the fear hypothesis was found by Spevack, Campbell,and Drake (1975), who reported that bilateral amygdalectomy produced substantial deficits in conditioned and unconditioned suppression. The lesioned rats consume reliably less water, so it seems unlikely that CER deficits can be attributed to their inability to suppress drinking. Amygdalectomy also failed to alter habituation, ruling out that hypothesis.

A more extensive study by Grossman, Grossman, and Walsh (1975) shed new light on the problem of amygdalar involvement

in avoidance learning. They found that bilateral electrolytic lesions in the periamygdalid piriform cortex of female rats markedly disrupted the acquisition of the two-way and one-way avoidance responses. Lesions in the six major subdivisions of the amygdala (cortical, medial, central, intercalated, lateral, and basolateral) consistently produce facilitory effects on active avoidance behavior (one- or two-way), but passive avoidance was impaired in animals with lesions in the central, intercalated, and basolateral nuclei. Since these results were quite different from those found by Ursin (1965) in the cat, the authors of this study suggested that species differences may exist between the cat and the rat in amygdalar function.

A problem arises when attempting to discuss studies based on amygdalar lesions, especially if the lesions are intended to involve only certain nuclei or subdivisions of the complex. The major nuclei are easily distinguishable in terms of their gross histological appearance (Lammers, 1972). However they are long thin structures with irregular boundaries that change in the mediolateral as well as the dorsoventral dimensions, which makes it essentially impossible to make a lesion by conventional procedures which affects only the major part of one nucleus. There is often significant involvement of several other nuclei as well as the surrounding tissue. Considering these circumstances it is not surprising that a number of studies disagree on particular

localization of effects.

However, the fear hypothesis appears to account for a large volume of the results in studies concerned with the effects of amygdalar lesions on avoidance behavior. It appears that fear may be mediated in part by amygdalar nuclei (or the surrounding area). Data presented here also tend to support the conclusion that the basolateral nuclei (or other surrounding structures) are concerned with inhibition of responding, accounting for why lesions in this area produce deficits in learning a passive avoidance task.

Statement of the Problem

In an effort to systematically examine the role of the CNS in the development of conditioned heart rate responses, the first step is to consider the behavioral paradigm on which the investigation will be based. It is apparent from the literature reviewed here that classical conditioning of heart rate has been conceptualized in a number of different ways, based on the variety of data that have been obtained. None of the theoretical frameworks reviewed offers a complete explanation of the available data, but a number of the considerations may be pertinent in interpreting neurophysiological data.

In the rat, the descending pathway mediating outflow that causes a decelerative heart rate CR has been shown to converge on motoneurons in the dorsal motor nucleus of the vagus and related brain stem structures (Nosaka et al, 1979).

Logical succeeding steps would be to determine the nuclei that project to this nucleus and then determine if lesioning them alters the normal development of the CR. If loss of a particular structure has an effect, then further analysis would be warranted.

The anatomical literature indicates that the amygdala (particularly the central nucleus) is a promising structure with which to begin such an investigation. Using auto-radiography, Hopkins and Holstege (1978) demonstrated that the amygdala sends projections to the rostrocaudal vagal nuclei (dorsal motor nucleus of the vagus and the nucleus tractus solitarius) in the cat. Schwaber et al (1980) further delineated the central nucleus of the amygdala as the source of these fibers in the rabbit.

Further evidence which enhances the possibility of finding a significant role for the amygdala in the development of conditioned heart rate responses comes from related behavioral investigations. Stimulation or ablation of the amygdala or its individual nuclei has been shown to produce changes in the orienting response, defense reactions, and fear responses.

Aim of this Study

The purpose of the present study was to examine the effects of regional bilateral lesions of the amygdala on heart rate during aversive differential classical conditioning using tone CSs and a shock US. One group of animals

received bilateral lesions in the amygdala and another group received sham lesions. After a 2-week recovery period a differential conditioning paradigm was presented to each animal. Ten habituation trials, thirty conditioning trials, and twenty extinction trials were given. Heart rate responses to the CSs and US were subtracted from the baseline measures and these scores were analyzed to determine whether or not the responses differed between sham-operated and lesioned animals.

METHODS

Animal Preparation

Twenty-four Long-Evans female rats (250-300 g body weight) obtained from Charles River Farms were used as subjects. Twelve were lesioned and 12 were sham-operated controls. Animals were anesthetized with sodium pentobarbital (IP, 40 mg/kg) and were supplemented as needed. Two incisions were made for inserting heart rate electrodes, one above the sternum on the ventral aspect and one above the vertebral border of the left scapula. The skin was freed from subcutaneous tissue and muscle using blunt dissection. A hemostat was pushed subcutaneously from each incision to the back of a midline scalp incision to grasp the star-washer end of a heart rate electrode and pull it underneath the skin to the incision site. The star-washer was sutured onto the underlying tissue and the incision closed. A stainless steel wire was placed in the temporal muscles for animal ground. Leads from the heart rate electrodes and the ground were soldered into a Microtech connector (DR-4S-4). Then each animal was mounted in a Kopf stereotaxic apparatus and the skull exposed. Using stereotaxic coordinates, two burr holes were drilled in the skull through which lesioning electrodes were directed. Stereotaxic coordinates were: AP: 5.8, L: $\pm$4.5, and V: -1.75 (Pellegrino, Pellegrino, & Cushman, 1979). Coordinates were referenced to stereotaxic zero and the incisor bar was

set at +5 mm. An electrode was used once and then discarded. An electrode was lowered to the target area and 10 min was allowed before lesioning. After lesioning there was a 10 min wait before the electrode was removed. The lesions were made bilaterally using a Radionics Radio Frequency Generator for delivering 5 mA for 1 min. A 20 ga needle inserted under the skin of the animal's thorax served as the cathode. The procedure was identical for the control animals, except no current was delivered through the electrode. Three stainless steel anchor screws were placed in the skull and dental acrylic was built up around the screws and the electrodes in the connector to form a head post. The incision was sutured shut around the connector. Topical antibacterial powder was applied liberally to all incision sites. All animals were allowed to recover 10-14 days before conditioning.

Electrodes

The lesioning electrodes were constructed from a 00 stainless steel insect pin insulated with Epoxylite eight times. The tapered tip was ground off so that the exposed surface was 0.25-0.30 mm in diameter. Heart rate electrodes consisted of a star washer soldered to a plastic-coated piece of 26 ga stranded stainless steel wire. The soldered connection was enclosed in dental acrylic.

Recording Apparatus

The heart rate signal was led from a cable connected

to the head plug out of the sound-attenuated chamber (Industrial Acoustics) and into a Tektronix Type AM 502 differential amplifier with variable gain. Data were collected in digital form on magnetic LINC tape by a PDP-12 computer.

Stimulation Apparatus and Parameters

Stimulus presentation was controlled by a Devices Digitimer which generated timed intervals and a time zero pulse when triggered by a film programmer at a variable inter-trial interval (average 2.5 min). Tones were used for both CS+ and CS- (9sec, 1K Hz (71 dB re 0.0002μ bar measured on the C scale) or 5K Hz (48 dB measured on the C scale)) and were produced by Tektronix Type AF 501 oscillators, amplified by a Southwest Technical products Power Amplifier 207-A, and delivered through a speaker mounted on the wall of the sound-attenuated chamber. A ventilation fan in the chamber was running during the experiment and produced a baseline noise level of 33 dB measured on the C scale. A shock US (1 sec, 1.3 mA) was used. It was produced by a Grason Stadler constant current stimulator (Model E6070B) and delivered through a pair of 20 ga hypodermic needles placed subcutaneously on either side of the rat's thoracic cavity. When a satisfactory heart rate signal could not be obtained from the implanted leads, heart rate was recorded from the 20 ga needles and the shock was switched into the circuit at the appropriate time. The delivery of the shock was controlled through a Coulbourn relay. A binary counter composed of

flip-flops determined when the relay would close so that shock was delivered only on the CS+ trials during the conditioning phase of the experiment.

Conditioning Procedures

During conditioning each animal was restrained in an inverted U-shaped tube restrainer 65 cm from the speaker in the sound-attenuated chamber. After a 30 min adaptation period, 10 trials of each CS alone were given. Then there were 30 trials of a delayed conditioning paradigm, with the US overlapping the last 1 sec of the CS+, interspersed with 30 trials of the CS- alone. The CS-US interval was 8 sec. An extinction period of 20 trials of each CS alone followed. In all of the above sequences the CS+ and CS- trials were delivered according to a randomized schedule, with not more than two consecutive presentations of a CS. The same schedule was used for all rats. One of the CSs was assigned randomly to each animal to be the CS+. The frequency of the tone that served as the CS+ was counterbalanced across groups.

Histology

After the experiment was complete, the animal was deeply anesthetized with sodium pentobarbital (IP, 40 mg/kg). A cardiac perfusion was performed using 10% formalin. The brain was blocked to contain the lesion site and stored in 10% buffered formalin until it was processed (frozen sections and Nissl staining). The stereotaxic atlas of Pellegrino et al (1979) was consulted in determining the actual location of

lesions.

Data Analysis

The occurrence of heart beats in relation to the time zero pulse was recorded by setting a Schmitt trigger to reliably sense the heart beat and exclude noise. A total of 25 sec of real time data was collected (8 sec pre-CS, 9 sec CS, and 8 sec post-US). The analysis of the CS-US interval was based on the following data. Ten-trial blocks were accumulated for each CS (one habituation, three conditioning, and two extinction). Four difference scores were computed for each of the six 10-trial blocks (mean rate during each of the four 2-sec counting periods* during the CS interval minus the mean rate during the 8 sec pre-CS interval**). These difference scores were analyzed using a 4-way analysis of variance (Factor A, lesion vs. control; Factor B, CS+/CS-; Factor C, blocks of trials; and Factor D, counting periods) with repeated measures on B, C, and D.

The post-US interval was analyzed to determine the nature of the response to shock. The analysis of the UR was based on the six 10-trial blocks of data for the CS+. Three difference scores were computed for each block (mean rate during each of three 2-sec counting periods (beginning 1 sec after the offset of shock) minus the mean rate during the 8 sec pre-CS interval**). These difference scores were analyzed in a 3-way analysis of variance (Factor A, lesion vs. sham; Factor B, blocks of trials; Factor C, counting

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*Each 2-sec counting period was only 1950 ms.

**The 8-sec pre-CS interval was only 7800 ms.

periods) with repeated measures on B and C.

The CS-US interval was analyzed during habituation to determine if there was an orienting response to either tone. These data were analyzed in blocks of 2 trials using the 10 trials in the habituation period for both frequencies. Four difference scores were computed for each of the five 2-trial blocks (mean rate during each of the four 2-sec counting periods* during the CS interval minus the mean rate during the 8 sec pre-CS interval**). These difference scores were analyzed in a 4-way analysis of variance (Factor A, lesion vs. sham; Factor B, 5K vs. 1 K; Factor C, Blocks of trials; Factor D, counting periods) with repeated measures on B, C, and D.

The mean rates in the pre-CS interval were analyzed to determine if the lesions resulted in any baseline differences in heart rate. The baseline scores for each 10-trial block were analyzed in a 2-way analysis of variance (Factor A, lesion vs. sham; Factor B, blocks of trials) with repeated measures on B.

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*Each 2-sec counting period was only 1950 ms.

**The 8-sec pre-CS interval was only 7800 ms.

RESULTS

Histology

The results of the histological analysis are summarized in Table 1. The anterior-posterior coordinate represents the section where the lesion was largest. The lesions produced were symmetrical in shape, so the extent of the lesion in the lateral plane is approximately equivalent to the extent of the lesion in the anterior-posterior plane. The animals were subdivided into 2 groups according to the size and location of the lesions. Five animals (L001, L005, L008, L009, L020) had large lesions which affected a significant part of both the basolateral and centromedial groups. Another group (L003, L006, L007, L010, L011) had smaller lesions primarily confined to the basolateral nuclei. Two other animals (L004, L015) had lesions which were not compatible with either group.

Heart Rate Responses

The CS-US interval was analyzed to determine whether or not conditioning occurred. The data for this analysis are depicted in Figure 1. It is evident from this graph that no patterns emerge which distinquish the lesioned from the sham-operated animals. Also, there is no observable change in the data over trials that would indicate an effect due to the conditioning procedures. These conclusions were confirmed by the statistical analysis. The 4-way analysis of variance resulted in one significant effect, i.e.,

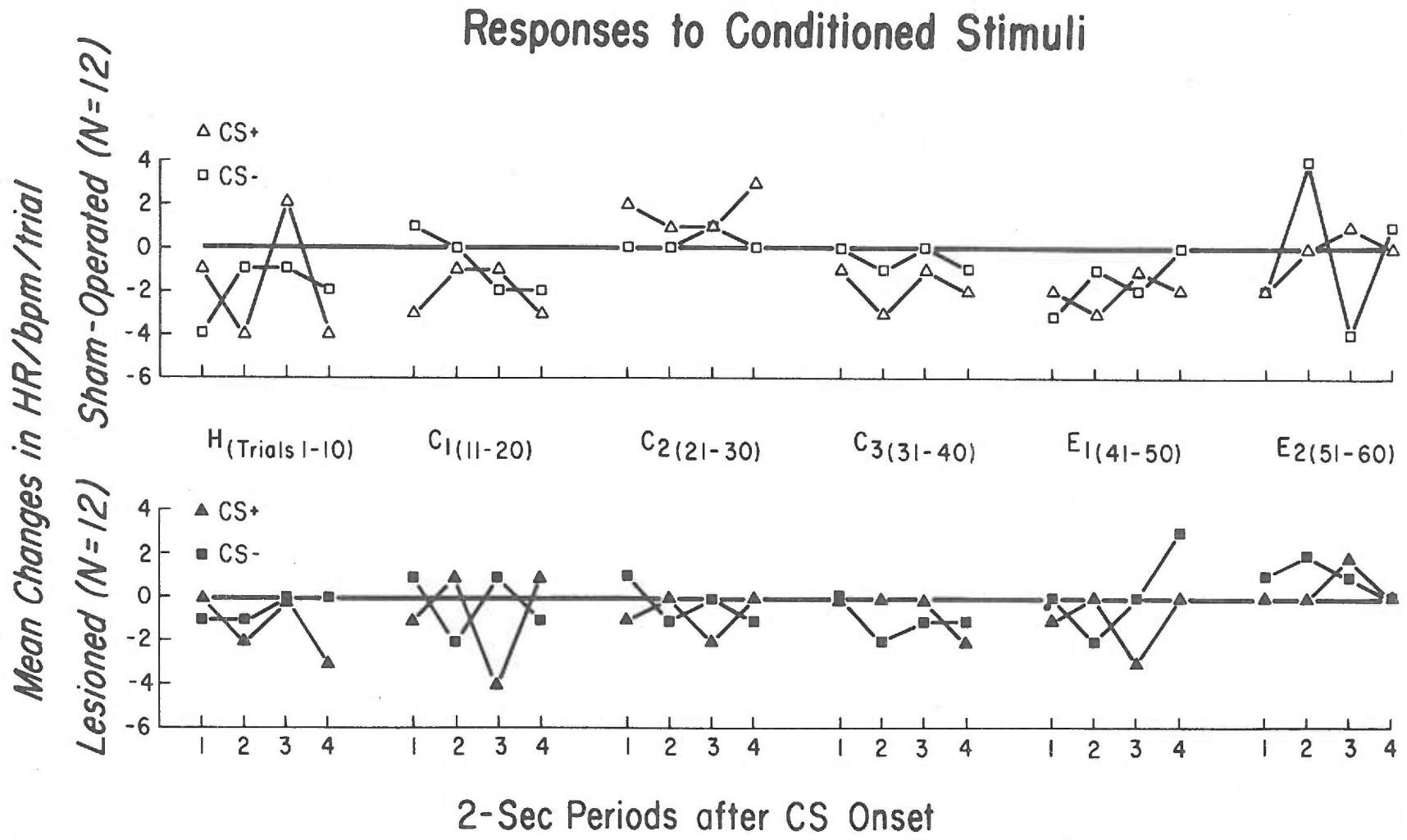
Table 1. Summary of Histological Results

Based on the stereotaxic atlas of Pellegrino, Pellegrino, and Cushman (1979).

	LEFT			RIGHT		
RAT #	AP	L	V	AP	L	V
(Animals whose lesions affected both the basolateral and centromedial groups of nuclei.)						
L001	5.4	2.0-4.5	-1.5--3.5	5.4	2.0-3.0	-1.5--3.5
L005	5.0	2.5-6.0	-1.0--2.5	4.6	2.5-6.0	-1.0--3.0
L008	4.8	3.0-6.0	-1.0--3.0	4.4	3.0-7.0	0.0--2.5
L009	5.0	3.0-6.0	-1.0--5.0	4.4	4.0-6.0	-0.5--3.0
L020	4.8	3.5-7.25	0.5--2.0	4.6	4.0-6.0	-2.0--3.75
(Animals whose lesions affected primarily the basolateral nuclei.)						
L003	4.2	4.0-6.0	-1.0--3.0	4.2	4.0-6.0	-2.0--3.0
L010	4.8	4.5-7.0	-1.5--3.5	4.4	4.0-6.5	-2.0--3.0
L007	4.8	4.0-6.0	-1.0--3.5	4.8	4.5-6.5	-0.5--3.0
L006	5.0	4.5-6.5	-1.0--3.0	5.0	4.0-6.0	-1.5--3.0
L011	5.4	4.5-7.0	-1.0--2.5	5.4	4.0-6.5	-1.0--3.0
(Animals whose lesions were not compatible with either group above.)						
L015	4.6	5.0-6.5	1.0-1.5	*	*	*
L004	3.8	5.25-7.5	-1.0--3.75	3.8	4.0-6.0	-0.5--2.0

*Unable to confirm lesion location due to loss of tissue sections.

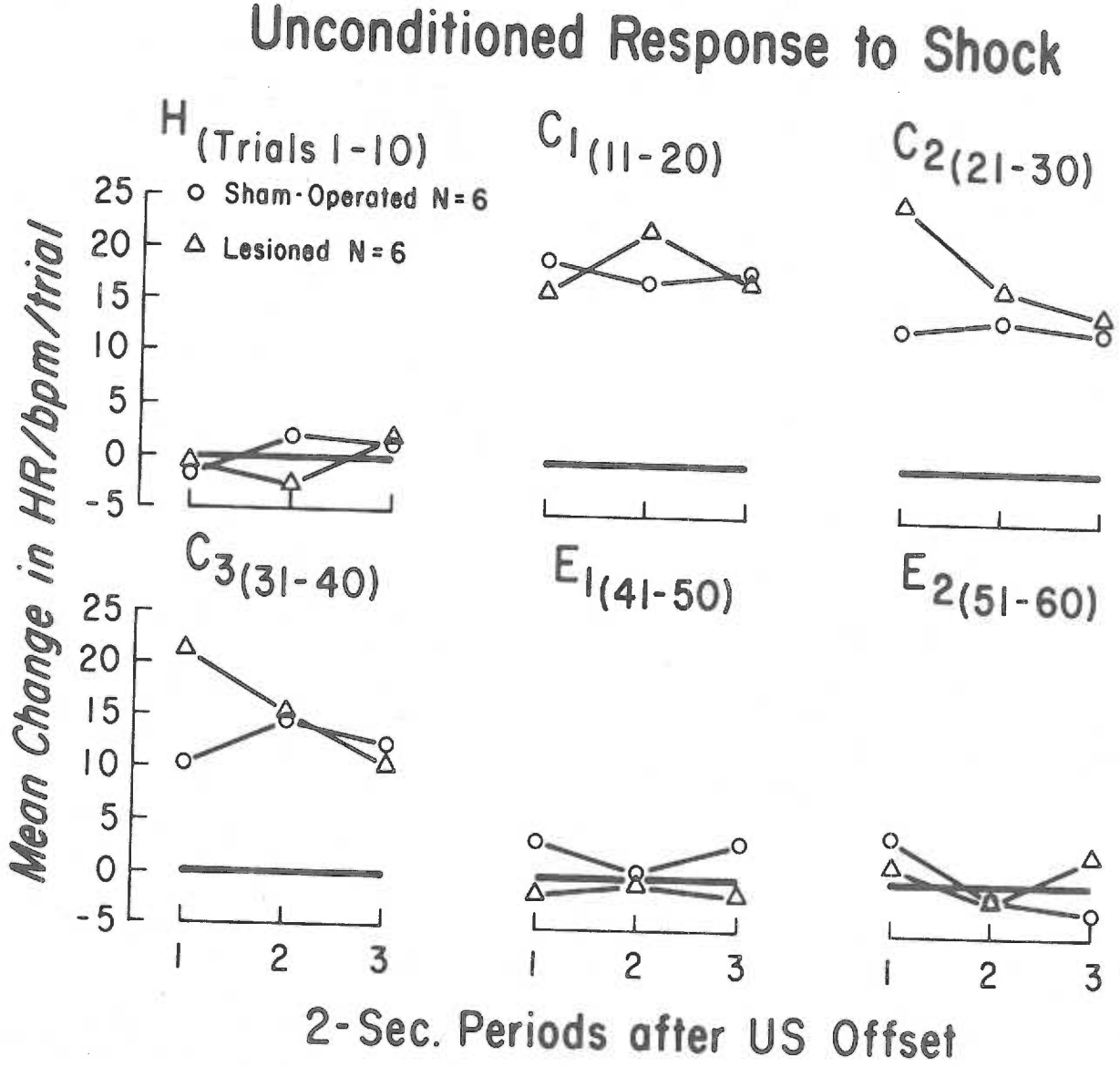
Figure 1. Mean heart rate response during the CS (CS period minus baseline) for the lesioned and sham-operated groups during four 2-sec periods of CS+ and CS- averaged over 10-trial blocks (one habituation (H), three conditioning (C), and two extinction (E). Note the absence of any orderly change in response to either the CS+ or CS- or any difference between them.


Responses to Conditioned Stimuli
Mean Changes in HR/bpm/trial
Sham-Operated (N=12)
Lesioned (N=12)
CS+
CS-
4
2
0
-2
-4
-6
H (Trials 1-10)
C1 (11-20)
C2 (21-30)
C3 (31-40)
E1 (41-50)
E2 (51-60)
1 2 3 4
2-Sec Periods after CS Onset

Factor C, blocks of trials, $F(5, 110) = 2.53$, $p < .05$. It should be noted that this effect involved very small beat per minute changes ($\pm$ 1.5). The Newman-Keuls test showed that the only significant difference was between the habituation score and the score for the second block of conditioning trials. A second analysis was done on the data with the lesioned animals divided into small and large lesion groups according to the histological results. This analysis resulted in no significant effects or interactions. A third analysis was done on the data from the conditioning blocks only. This analysis resulted in no significant effects or interactions, too.

In the analysis of the post-US period (to investigate the nature of the UR to shock), only six animals in each group provided enough data free of artifact to be included in the analysis. The 3-way analysis of variance on the unconditioned response to shock yielded an A x B x C interaction, $F(10, 100) = 2.43$, $p < .05$. (The data are depicted in Figure 2.) The follow-up analyses indicate that for the sham-operated animals there was a significant B effect, blocks of trials, $F(5, 25) = 9.36$, $p < .001$. The Newman-Keuls test showed that the only significant difference was between the score for the first block of conditioning and the score for the second block of extinction. For the lesioned animals the subsequent analysis of variance yielded a significant B x C interaction (B, blocks of trials; C, counting periods),

Figure 2. Mean heart rate response during post-US period (US period minus baseline) for lesioned and sham-operated groups during three 2-sec periods averaged over 10-trial blocks (one habituation (H), three conditioning (C), and two extinction (E)). Note that the shock does produce cardioacceleration in both groups, but the pattern is slightly different for the lesioned and the sham-operated animals.

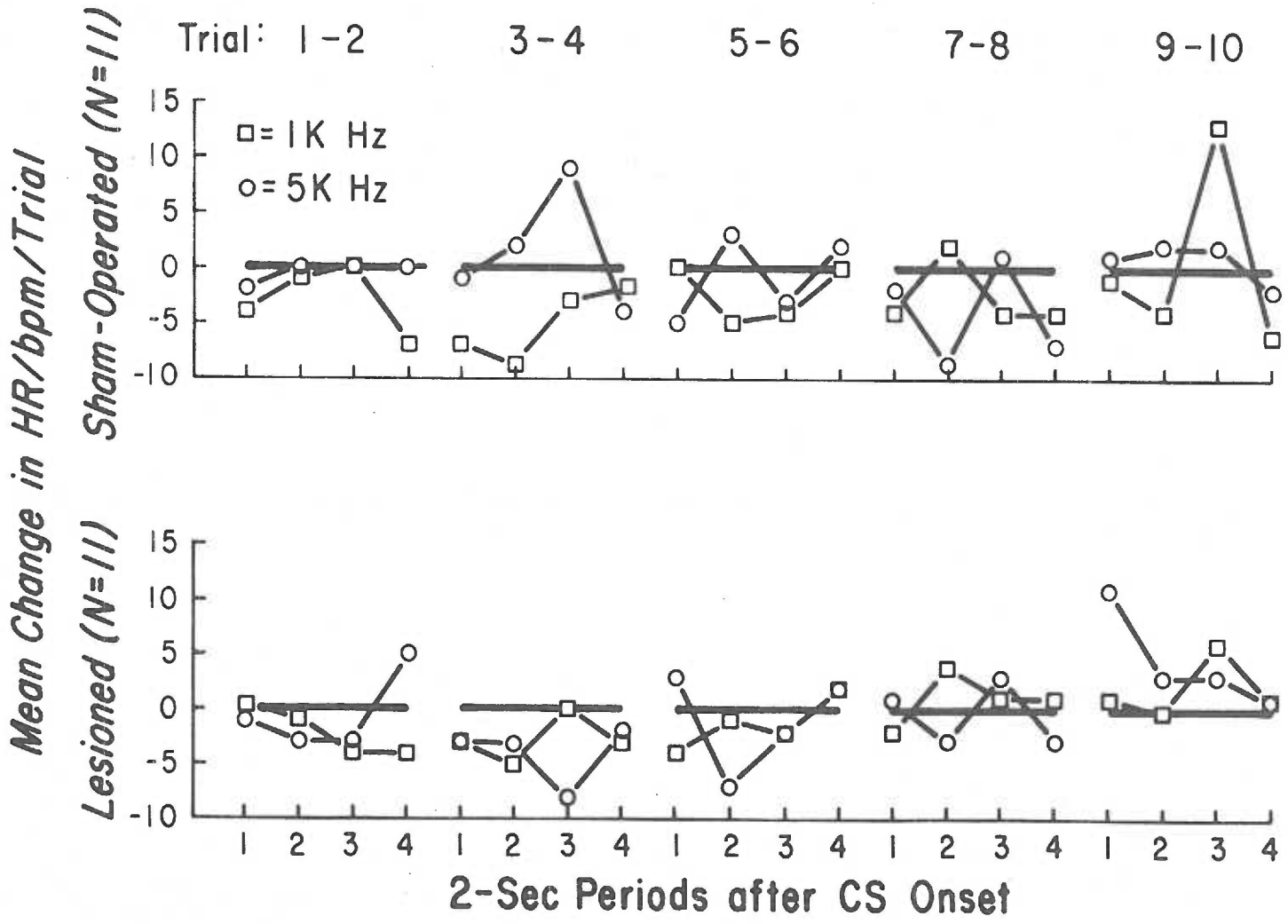

Unconditioned Response to Shock
H (Trials 1-10)
C1 (11-20)
C2 (21-30)
C3 (31-40)
E1 (41-50)
E2 (51-60)
Sham-Operated N=6
Lesioned N=6
Mean Change in HR/bpm/trial
25
20
15
10
5
0
-5
1
2
3
2-Sec. Periods after US Offset

$F(10, 50) = 2.18$, $p < .05$. Follow-up one-way analyses of variance indicated that the responses changed across trials in all three counting periods (for the first counting period, $F(5, 25) = 7.1$, $p < .01$; for the second counting period, $F(5, 25) = 6.9$, $p < .01$; for the third counting period, $F(5, 25) = 3.9$, $p < .05$). The results of the Newman-Keuls tests indicate that for the first two counting periods the response in all conditioning trials was different from the responses in habituation and extinction and were, therefore, probably due to the presence of shock. In the third counting period the response in the second block of conditioning trials was only significantly different from the response in the first extinction block. In summary it appears that the lesioned animals were more responsive to the shock stimulus than the sham-operated animals, especially in the first two counting periods.

The data from the CS-US interval during habituation are depicted in Figure 3. These data were analyzed to determine if there was an orienting response to the tones. One sham-operated animal was dropped from the analysis because of excessive artifact in the data. An animal from the lesioned group was eliminated at random in order to retain equal numbers for the analysis. There was no apparent pattern of responding that can be related to the initial trials of the CSs alone. In addition there are no obvious differences between the lesioned and sham-operated animals. The 4-way analysis

Figure 3. Mean heart rate response (CS period minus baseline) during four 2-sec periods of the 5K Hz tone and 1 K Hz tone averaged over 2-trial blocks. Note the absence of any orienting response to either tone in either group, or any differences between groups.

Response to Tones during Habituation



of variance confirmed these observations; no significant effects or interactions were found.

The analysis of the baseline data resulted in no significant effects or interactions, indicating that neither the lesions nor the conditioning paradigm resulted in consistent changes in baseline heart rate.

DISCUSSION

Responses to Conditioned Stimuli

The lack of any changes in heart rate attributable to the differential classical conditioning paradigm was a surprising result. Martin and Fitzgerald (1980), using a differential conditioning procedure, found that restrained rats showed heart rate deceleration to both paired and unpaired CSs, with the magnitude of decelerations being significantly larger to the CS paired with shock. In addition a number of studies using between-subjects controls have demonstrated decelerative heart rate CRs in physically restrained rats receiving a shock US delivered to the chest, tail, or footpads in a Pavlovian conditioning situation (Fitzgerald & Martin, 1971; Fitzgerald et al, 1973; Fitzgerald & Teyler, 1970; Holdstock, 1970; Holdstock & Schwartzbaum, 1965; Teyler, 1971).

It is unclear what one should expect to find if the rats did not distinguish between the two tones and were for that reason on a 50 percent reinforcement schedule. Some evidence would suggest that there would be no difference in the terminal levels of conditioned responding; so one would predict a decelerative CR comparable to that seen in studies with between-subjects controls (Fitzgerald, Vardaris, & Teyler, 1966; Slivka & Bitterman, 1966). On the other hand other studies that have used partial reinforcement with classical conditioning have indicated that the asymptotic level of conditioning would be reduced (Berger, Yarczower,

& Bitterman, 1965; Wagner, Siegel, & Fein, 1967).

The failure to detect a reliable orienting response to the initial presentation of tones was an even more unusual finding. The literature supporting the reliability of a decelerative heart rate response to the initial presentation of an auditory stimulus in the rat is extensive (Black, 1964; Cunningham et al, 1977; Fitzgerald & Hoffman, 1976a, 1976b; Fitzgerald & Martin, 1971; Fitzgerald et al, 1973; Fitzgerald & Teyler, 1970; Hoffman, 1977; Martin & Fitzgerald, 1980; Stainbrook, 1975; Stern & Ward, 1961; Teyler, 1971; Vardaris, 1971). Black (1964) reported that for unrestrained rats, the magnitude of deceleration increased with the duration of a white-noise stimulus and after five trials changed to acceleration. Stern and Ward (1961) used restrained rats and observed that the decelerative OR to a "house bell" habituated to near zero after five trials. In the remaining studies done in Fitzgerald's laboratory, the OR was consistently a monophasic deceleration averaging 5 to 40 beats per min and habituated to near zero within the first 5 to 15 stimulus presentations.

Responses found in the present study were not monophasic and the mean change was less than 3 beats per min during the first presentation of the tone for both groups. It was this result taken in the context of the above precedent which prompted a further examination of the effect of breaking the tympanic membranes during stereotaxic surgery on hearing in

the rat. Originally it was not suspected that this procedure would result in a large enough loss to interfere with the animals' reception of the auditory stimuli. The sound level of the tones used was compared with the behavioral hearing curve of Kelly and Masterton (1977); it was found that the tones used as experimental stimuli were in excess of the auditory threshold of the albino rat by at least 60 dB (re 20μ N/m^2 relative to the absolute reference level). Such a loss was not anticipated by several authors who have written procedures for stereotaxic surgery. Skinner (1971) advises the reader to "disregard any bleeding from the ear, for the ruptured ear drums will heal" and then approximates complete recovery within 4-7 days. In similar texts Cooley & Vanderwolf (1978) and Hart (1969) failed to mention either the potential for damage or possible recovery. In addition several authors report responding to tones or clicks in lesioned and sham-operated animals, making no mention of precautions to avoid damage to the tympanic membranes (Holdstock, 1969; Plunkett, 1979; Reyes-Vazquez, Ibarra, & Brust-Carmona, 1979). However following this experiment, a bar-press study was designed to determine if the animals' hearing was impaired by this procedure even after the 2-week recovery period (See Appendix). The animals were trained to bar press for food and then food was available on a variable interval schedule signalled by a tone. The results of this study indicated that breaking the tympanic membranes resulted in a serious hearing impairment.

It appears that the animals had not recovered adequately from this loss in the 2-week recovery period to expect successful conditioning to auditory stimuli.

In conclusion the loss of hearing suffered by these animals is sufficient to account for their lack of orienting response to the tones during their initial presentation, as well as the failure to develop conditioned responses to the CS+. Considering this sensory deficit and lack of statistical differences between lesioned and sham-operated animals during the various phases of the experiment, it seems unwise to make any conclusions about the effects of amygdalar lesions on orienting or conditioned responses.

Responses to Unconditioned Stimuli

In this study, the unconditioned response to shock was an acceleration for both lesioned and sham-operated rats, which is consistent with a number of previous studies in both restrained and unrestrained rats (Fitzgerald, Vardaris, & Brown, 1966; Holdstock & Schwartzbaum, 1965; MacDonald, Stern & Hahn, 1963). Kapp et al (1979) demonstrated that rabbits with large lesions producing damage to the central nucleus of the amygdala showed changes in the topography of the cardioaccelerative heart rate UR which were not present in sham-operated controls. The peak magnitude of the UR remained unaltered, but the duration of the acceleratory response was prolonged and showed less habituation over trials in the lesioned animals. There are tendencies in the data

presented here which indicated that the UR in the lesioned animals occurred more consistently to the presentation of shock than in the sham-operated animals. These results suggest that amygdalar damage may produce effects on the cardiovascular response to unconditioned stressful stimuli. Concerning these results, it is important to consider that the direction and magnitude of heart rate URs has been linked to gross movement in the rabbit (Schneiderman, 1974). Also, Schwartzbaum and Gay (1966) reported that animals with amygdalar lesions showed increased activity in an open-field test. However, since additional observations of general motoric reaction to shock were not made in this study, one can only speculate whether the lesion effects on the heart rate UR were secondary to alterations in motoric reactivity to shock.

There have been a number of other effects attributed to amygdalar lesions which would be consistent with this change in responding to the UR. Amygdalar lesions have usually resulted in reduced responsiveness to normally noxious stimuli, but several cases of hyperemotionality have also been reported. For example, Wood (1958) found in the cat that small lesions in the basal or central nuclei of the amygdalar increased responsiveness. It is possible that subtotal lesions such as those may considerably alter the reactivity of remaining amygdaloid nuclei. It appears that differences in species, surgical techniques, size of lesion, involvement of different extra-amygdaloid structures, and pre- and post-experience

cannot be considered as complete explanations for the different response patterns. Schreiner and Kling (1953) and Kling, Orbach, Schwartz, and Towne (1960) have produced rage in a small number of cats using exactly the same techniques as those which produced placidity in other cats. Furthermore they were unable to find any histological differences in these animals that correlated with behavioral differences.

More recently there has been interest in rating lesioned animals' emotionality based on criteria such as response to touch, resistance to capture and handling, and vocalization, urination, and defecation during capture and handling. Several reports indicate that there is decreased or unchanged affective behavior in rats with amygdalar lesions, particularly within a week after surgery (e.g., Schwartzbaum & Gay, 1966). However some reports indicate that animals may overcome this"post-operative depression". At this point they often exhibit increased reactivity to environmental stimuli and do not appear to habituate to stimulus events (Kleiner, Meyer, & Meyer, 1967; Schwartzbaum & Gay, 1966). Such an alteration in response pattern could account for the more consistent responding to the unconditioned stimuli in the present experiment.

Douglas and Pribram (1966) developed a theory around several experiments which indicated that the amygdalar may play some role in allowing an experience to be registered. According to this theory, when the amygdala is damaged,

experience is not registered and cannot habituate. Cormier (1981) has elaborated another theory in which the amygdala is responsible for the processing of external unconditioned stimuli by augmentation of neural and endocrine response to them. This augmentation leads to the creation of a mood state that makes certain species-specific behaviors more likely to occur. When the destruction of the amygdala interrupts this process by inhibiting the formation of an appropriate mood state, inappropriate responding may result. The finding in this experiment, that the lesioned animals responded differently to the shock US than the sham-operated control, is consistent with this theory.

Based on these data, it seems reasonable to postulate a role for the amygdala in modulating the emotional responding to noxious stimuli, but the delineation of this mechanism will require further study due to the complexities of the available data.

SUMMARY AND CONCLUSIONS

The present study was designed to determine if lesions in the amygdala affected the nature of conditioned and unconditioned heart rate responses using a classical conditioning paradigm. Heart rate responses to both the conditioned (tones) and unconditioned (shock) stimuli were recorded during conditioning, habituation, and extinction.

There were no significant differences between the heart rate responses to the CS+ and CS- during the conditioning period in either the lesioned or sham-operated group. In addition there was no evidence of an orienting response in the heart rate to the initial presentations of either tone in either of the two groups.

These findings led to further investigation of the practice of breaking the tympanic membrane to insure correct placement of a rat in the stereotaxic apparatus. The results of a bar-press study suggest that this procedure results in serious impairment of the animal's hearing, which is not regained in a 2-week recovery period.

There did appear to be some difference in the heart rate response pattern to shock between the lesioned and sham-operated groups. Judging from the analysis of the data, it appears that the lesioned animals are more responsive to the shock than the sham-operated animals. Other studies have shown similar alterations in heart rate responding after amygdalar lesions. These data would support the conclusion

that the amygdala or some subset of its nuclei have a role in modulating emotional responding to noxious stimuli.

APPENDIX

PURPOSE

One procedure for insuring proper placement of the ear bars when mounting a rat in a stereotaxic apparatus, is to break the tympanic membranes. The purpose of this experiment was to determine if that procedure impairs the animal's hearing. Also, whether there is sufficient recovery of that faculty after two weeks to expect successful conditioning to auditory stimuli.

METHOD

Subjects

Five Long-Evans female rats (250-300 g body weight at the beginning of the experiment) were used as subjects. Two of these subjects had been used as lesioned animals in the previous experiment. They were chosen because their body weight was in a normal range for their age. If they had suffered a loss of appetite from the amygdalar lesions, it had been recovered. Two of the animals were prepared in the same way as the sham-operated controls in the previous experiment, but did not receive the classical conditioning regimen. They were allowed to recover for at least 2 weeks before beginning this experiment. Both the lesioned and sham-operated animals had their tympanic membranes broken when they were placed in the stereotaxic apparatus. The fifth subject was a control, which was not subjected to any surgical or behavioral procedures prior to this experiment.

Apparatus

This experiment was performed in a bar press apparatus (Grason-Stadler box and Ralph Gerbrands Company bar and feeder) in a sound-attenuated chamber (Industrial Acoustics). Stimulus presentation and food availability were controlled by a Devices Digitimer, which generated a timed interval when triggered by a film programmer and closed a relay for that period allowing the two events to occur. A tone was used as the discriminative stimulus (20 sec, 1 K Hz, 71 dB re 0.0002μ bar on the C scale, measured from the locus of the rat's ear 65 cm from the speaker). It was produced by a Tektronix Type AF 501 oscillator, amplified by a Southwest Technical Products Power amplifier 207-A, and delivered through a speaker mounted in the wall of the sound-attenuated chamber. Bar presses were recorded on two Coulbourn cumulative counters, one was activated during the tone and one when it was absent.

Procedure

All five subjects received only one small piece of food (4-5 g) each day for 7 days. Water was available ad lib. In most cases this procedure was sufficient to reduce the animal's weight to 80% of its original body weight. If not, the same procedure was continued on successive days until that goal was achieved. The animals were maintained at or below 80% of their body weight for the remainder of the experiment.

On the first day of the experiment the animals were magazine trained. Shaping was used as needed and food was available on a continuous reinforcement schedule. Each animal was allowed to bar press for food 50 times and then removed to its home cage.

For the next 6 days each animal was put into the apparatus for 1 hour. Bar presses were reinforced with food only during the tone, which was presented on a variable inter-trial interval ($\overline{X}$ = 2 min). On the seventh day the control animal was anesthetized with ether and placed in the stereotaxic apparatus (breaking the tympanic membranes of both ears) and then removed. This animal received an additional day of training on the following day. One of the sham-operated animals was given 2 additional days of training. The first day was identical to those described above. On the second additional day food was available on the same schedule, but there was no tone to signal those periods.

RESULTS

The results of the experiment are summarized in Table 2. The scores listed in the table represent the percentage of reinforced bar presses (i.e., (Number of bar presses during the tone/ Total bar presses) x 100). It is apparent from the data that by the fourth day of training the control animal was pressing primarily when the tone was on. Placement in the stereotaxic apparatus on the 7th day appeared to eliminate the animal's ability to discriminate when food

Table 2. Percentage of Reinforced Bar Presses during a Discrimination Task

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8
Control	6	32	57	82	81	84	3***	
Lesioned (1)*	9	6	5	7	6	6		
Lesioned (2)*	6	6	6	7	6	8		
Sham-operated (1)**	13	12	7	5	17	5		
Sham-operated (2)**	5	8	7	10	10	19	23	11****

* Lesioned animals had recovered from surgery 26 days by Day 1 of the bar-press experiment.

** Sham-operated animals had recovered from surgery 16 days by Day 1 of the bar-press experiment.

*** After being placed in the stereotaxic apparatus.

**** With no tone to signal availability of reinforcement.

would be available, since it reduced substantially the number of bar presses and the animal's ability to get food reinforcement by pressing the bar at the appropriate time. The performance of the lesioned animals and the first sham-operated animal did not show any consistent improvement during the course of the experiment. The second sham-operated animal did appear to be improving its performance on the sixth day, so a seventh day of training was given. The animals performancedid not continue to show remarkable improvement. On the eighth day when no tone was present to signal the availability of reinforcement, performance declined to some extent.

CONCLUSIONS

Based on the data collected from the control animal, it appears that breaking the tympanic membranes in the rat results in a serious hearing impairment. These results do not indicate that the recovery of hearing within 2-3 weeks is reliable enough to expect successful conditioning to auditory stimuli.

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