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Neural correlates of cardiac orienting response habituation

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Introduction

In an animal's interactions with his environment, detection of, and responsiveness to, changes in stimulation are of unquestionable adaptive significance. Such changes in stimulation may involve the appearance of a stimulus, a change in the pattern of impinging stimulation, or a change in some physical parameter of an iterative stimulus. Of equal adaptive significance is the decrement in responsiveness to a stimulus change which has occurred repeatedly without acquiring signal value for the animal. The animal can "ignore" the redundant stimulus, responding instead to other stimuli of potential significance. This phenomenon, usually referred to as habituation, cannot be attributed to factors such as receptor adaptation, motor fatigue, or the absence of an overt response (Thompson & Spencer, 1966). An introspective examination of human habituation to repetitive stimulation suggests that some degree of sensory alteration is involved. An individual will note that he was "not aware" of a loudly ticking clock until its presence was mentioned to him. Two people may carry on a conversation in a crowded room apparently unaffected by the surrounding noise. Habituation can be shown to occur in a large number of species, ranging from planaria to man (Ratner, 1970). In fact, it has been considered to be one of the oldest, perhaps most rudimentary, forms of behavioral plasticity (Groves & Thompson, 1970; Ratner, 1970; Thorpe, 1963). Despite much theorizing and considerable research,

the neural mechanisms underlying this form of behavioral alteration have yet to be determined.

Thompson and Spencer (1966) have provided the most complete and general definition of habituation. Their definition consists of nine characteristics drawn from an extensive review of the literature on behavioral habituation:

1) Given that a particular stimulus elicits a response, repeated applications of the stimulus result in a negative exponential function of the number of stimulus presentations.
2) If the stimulus is withheld, the response tends to recover over time.
3) If repeated series of habituation training and spontaneous recovery are given, habituation becomes successively more rapid.
4) Other things being equal, the more rapid the frequency of stimulation, the more rapid and/or more pronounced is habituation.
5) The weaker the stimulus, the more rapid and/or more pronounced is habituation. Strong stimuli may yield no significant habituation.
6) The effects of habituation training may proceed beyond the zero or asymptotic response level.
7) Habituation of response to a given stimulus exhibits stimulus generalization.

8) Presentation of another (usually strong) stimulus results in recovery of the habituated response (dishabituation).

9) Upon repeated applications of the dishabituation stimulus, the amount of dishabituation produced habituates.

These characteristics were based upon habituation of a large number of types of responses in intact animals and experimental preparations; e.g., orienting responses (OR), startle responses, rostrotary nystagmus, and spinal reflexes. Thompson and Spencer (1966) noted the importance of using stimulus conditions and response systems which can be shown to exhibit these parametric features of habituation in any systematic exploration of the neural correlates of habituation.

Responses which show habituation range from early orienting (appetitive responses) evoked by potentially relevant stimuli to the consummatory responses evoked by an adequate stimulus (Ratner, 1970). In this context, the "goodness of fit" of Thompson and Spencer's definition is determined by the kind of response elicitation employed. For example, early in the orienting-consummatory continuum a wide variety of stimuli evoke responses which show the characteristics of habituation with repeated stimulus presentations. At the consummatory end of the continuum the range and possible variability of eliciting stimuli for a particular response narrows, and it becomes more difficult to specify or characterize habituation. The orienting response (reflex), first described by Pavlov (1927), has been widely used as an indication of stimulus detection (Thompson and Shaw, 1965).

It is a generalized response to stimulus change consisting of autonomic, behavioral, and electroencephalographic components which have been characterized under a variety of stimulus conditions in numerous species. Detailed descriptions of the response components may be found in the work of Davis, Buchwald, and Frankmann (1955), and Sokolov (1960; 1963; 1965). Reviews by Lynn (1966) and Razran (1961) also contain descriptions of the OR components and their interrelations. OR habituation studies provided a large part of the data for the general definition of habituation which has been outlined in the preceding pages.

Investigations of the neural mechanisms underlying habituation have seldom involved actual correlations between autonomic, somatic, or behavioral measures of habituation and underlying neural response changes. Instead the primary emphasis has been on neural response changes as a function of stimulus repetition, per se. Information of this kind may be relevant to the question of what mechanisms underly habituation. However, unless the occurrence and time course of habituation to the repetitive stimulus can be specified, it is difficult to relate any neural response alterations to the process of behavioral habituation.

Several theories suggesting mechanisms for habituation have been proposed. Hernandez-Peon (1960) suggested that habituation represents a decrease in sensory responsiveness, probably mediated by primary afferent inhibition. Sokolov (1963) hypothesized that transmission

of primary sensory input is not impaired as a function of stimulus repetition. He proposed that reticular afferent augmentation of primary sensory input mediates orienting to a novel stimulus, and that habituation reflects a decrement in this augmentation. Recently, Groves and Thompson (1970) have proposed that the synaptic properties of neuron populations within the primary S-R pathway mediating a response interact with the "state" system of the animal to produce the phenomena characteristic of habituation. The "state system" is defined as secondary afferent and efferent systems such as reticular pathways.

An adequate evaluation of existing theories requires first) the selection of a response pattern which shows the characteristics of habituation, second) the recording of quantifiable neural responses over successive stimulus presentations and test conditions, and third) a careful examination of correlations between characteristics of response habituation and neural response patterns over the same time period. The studies described in this paper were designed toward this end. Auditory stimuli which evoke the cardiac component of the OR were presented repeatedly. Tests for response recovery and subsequent habituation to a stimulus change were included to insure that changes in neural responses followed the typical pattern of cardiac response habituation. Multiple unit activity was recorded from neural sites which have been implicated in habituation mechanisms, and response variations following the time course of autonomic response

habituation were examined. Controls for peripheral gating factors such as middle ear muscle tonus variations and animal position in the sound field were used.

This study represents an attempt to determine some of the neural correlates of habituation to repetitive auditory stimulation, and to relate those correlates to the existing theories concerning habituation mechanisms. As the following literature review will reveal, a well controlled correlational study dealing with habituation to auditory stimulation is needed.

Literature survey

Approaches to the study of habituation have typically taken one of two general strategies. Some investigators have attempted to find correlations between the behavioral response decrements characterizing habituation and some pattern of neural response changes. Other studies have been designed to investigate neural response changes accompanying reflex habituation in more restricted neural systems (Thompson & Spencer, 1966).

More often than not experimenters taking the first strategy have expressed the intent of correlating behavioral habituation and neural response changes, but have actually limited their investigations to a search for neural response decrements occurring as a function of repetitive stimulation, per se. In fact, the relationship between neural response decrements and the course of behavioral habituation has typically received little comment and no systematic analysis (Hall, 1968; Kitzes & Buchwald, 1969; Marsh, McCarthy, Sheatz & Galambos, 1961; Webster, 1971; Worden & Marsh, 1963). This limitation of "correlational" habituation studies has been repeatedly noted in the literature (Groves & Thompson, 1970; Marsh & Worden, 1964; Thompson & Spencer, 1966; Weinberger, Goodman, & Kitzes, 1969); but it has continued to be ignored in experimental designs. An exception is Saffran's (1969) analysis of the auditory multiple unit response patterns associated with habituation of the rat's orienting response to auditory stimulation.

Typical of the second experimental approach are the hindlimb flexion habituation studies of Groves and Thompson (1970), investigations of the gill withdrawal reflex in Aplysia (Castellucci, Pinsker, Kupfermann, & Kandel, 1969), and studies of the fast flexor muscle habituation in the crayfish (Bruner & Kennedy, 1970). An investigation of the auditory system response changes accompanying habituation of sound-elicited eye movement is also representative of the "simple system" approach (Weinberger, Goodman, & Kitzes, 1969). In all of these studies the similarity of the course of habituation of the reflex under study to other instances of behavioral habituation was first established. Then, a single unit or evoked potential study of the relevant neural system provided information regarding possible mechanisms underlying the reflex habituation.

Groves and Thompson's (1970) experiments are perhaps the best example of this approach. They first established the similarity of the hindlimb flexion habituation in the acute spinal cat to startle response habituation in the intact rat. They then did a single unit analysis of response changes in the afferents, efferents, and interneurons involved in the cat handlimb flexion reflex during the course of response habituation. The generality of their findings to more complex stimulus-response systems has yet to be demonstrated. However, a potentially relevant experiment by Weinberger, et al. (1969) represented an attempt to study auditory evoked potential (EP) changes closely correlated with an acoustic eye movement habituation.

Additional studies in which careful attention is given to the relationship between quantitative measures of behavioral habituation to auditory stimuli and neural response modifications are in order. This kind of analysis should provide more useful information on the mechanisms underlying habituation than the previous analyses of neural responses as a function of stimulus repetition, per se.

Most of the recent work on the neural mechanisms of habituation can be related to one of three theoretical formulations. 1) The primary afferent inhibition hypothesis suggests that habituation is a function of reduced sensory input. The reduction is attributed to the effects of inhibition at the peripheral afferent synaptic junctions. 2) The stimulus-model comparator hypothesis assumes that habituation reflects a decrease in reticular augmentation of sensory input. 3) The dual process theory is based upon the assumption that some intrinsic synaptic mechanism must account for the response changes characterizing habituation. The plasticity of interneuronal transmission in the primary sensory afferent pathways is assumed to interact with that of the "state system" of the organism to yield habituation.

Primary afferent inhibition

The case for a primary afferent inhibition explanation of habituation was initially presented by Hernandez-Peon (1960). He reviewed a series of studies from his laboratory showing decrements in auditory EP's to a stimulus which had been repeatedly presented. These decreases in amplitude of the EP late components were shown to

occur in the cat's cochlear nucleus, inferior colliculus, medial geniculate, and in the auditory cortex. The idea that sensory input inhibition might underly behavioral habituation prompted numerous attempts to verify Hernandez-Peon's findings. Marsh, et al. (1961) reported amplitude decreases in the cochlear nucleus and auditory cortex following prolonged exposure to a repetitive stimulus. Other experimenters found such decrements in inferior collicular EP's (Dunlop, Webster, & Day, 1964; Dunlop, McLachlan, Webster, & Day, 1964). In subsequent experiments Worden and Marsh (1963) were unable to replicate their earlier demonstrations of EP amplitude decreases in the cochlear nucleus. They concluded that uncontrolled variation due to the animal's position in the sound field, changes in arousal level, etc. could either mimick or obscure the habituation effect. An experimental test of this hypothesis with appropriate controls revealed EP response decrements only in the auditory cortex (Marsh & Worden, 1964). Cochlear nucleus responses did not change in a consistent manner. At present there are in the literature several reports of cochlear nucleus evoked potential decrements occurring as a function of repetitive stimulation (Kitzes & Buchwald, 1969; Webster, 1971; Webster, Dunlop, Simons, & Aitkin, 1965). There are also reports of the absence of such decrements (Hall, 1968; Wickelgren, 1968).

Recording data from the inferior colliculus during repetitive stimulation presents an equally confusing picture. Hall (1968) and Wickelgren (1968) failed to find decreases in EP amplitude over

successive blocks of trials, while Webster and Bock (1971) and Webster (1971) reported decrements. Multiple unit response decreases have also been reported (Kitzes & Buchwald, 1969; Saffran, 1969). Decreases in amplitude of medial geniculate EP's have been a relatively consistent finding in experiments with adequate controls for animal position, arousal level, etc. (Hall, 1968; Kitzes & Buchwald, 1969; Webster, 1971; Wickelgren, 1968). Similarly, the amplitude of the late component of the auditory cortical EP has been shown to decrease with repeated stimulus presentations (Hall, 1968; Marsh & Worden, 1964; Wickelgren, 1968).

With the exception of a few general behavioral observations (Hernandez-Peon & Scherrer, 1955; Wickelgren, 1968), the reports of response changes in the primary sensory afferents have not been related to habituation of any behavioral, autonomic, or somatic response. Stimuli were presented at rates of 1 per 10 seconds, or faster, and over periods of hours, sometimes days. The EP's were averaged over large blocks of trials; consequently, the response decrements reported were changes occurring after hundreds of trials, rather than within the first 10 - 15 stimuli when behavioral habituation would probably have occurred. Data from the two experiments in which behavioral or reflex habituation was quantitatively measured indicated that primary afferent inhibition is probably not closely correlated with habituation. Saffran (1969) found inferior collicular and medial geniculate multiple unit activity (MUA) response decrements occurring 50 - 100 trials after

habituation of the rat immobilization response. Weinberger, et al. (1969) failed to find a correlation between EP changes in the lateral lemniscus and eye movement habituation.

Reasons for the lack of data on behavioral-neural correlations in habituation studies are probably both theoretical and procedural. Experimenters seem to have assumed that because habituation occurs as a function of stimulus repetition the neural correlates of stimulus repetition should serve to elucidate habituation mechanisms. As Thompson and Spencer (1966) have demonstrated, habituation must be specified in terms of parameters other than just stimulus repetition. The procedural reason for the absence of behavioral and neural response correlations probably lies in the practicality of averaging EP's over blocks of trials rather than looking at changes occurring over the initial sequence of stimulus presentations. Averaged evoked responses (AER) for an initial block of trials have often been compared with AER's for a later block (Hall, 1968; Marsh & Worden, 1964; Wickelgren, 1968). This process may obscure any changes occurring over the first 10 - 20 stimulus presentations. It is during this period that most behavioral indications of habituation develop (Lynn, 1966). Recently, the technique of studying trial by trial response variations in MUA recordings has been found to be a useful method of evaluating neural response changes (Kitzes & Buchwald, 1969; Saffran, 1969). MUA responses reflect evoked changes in the background frequency of firing of the pool of neurons surrounding a small macroelectrode. These are,

of course, net changes including unit responses which consist of decreases as well as increases in level of spontaneous activity. MUA recordings are made possible by filtering out slow potential changes between the recording and reference electrodes, amplifying only high frequency activity (>550 Hz.). The characteristics of the resulting activity (pulse widths, pulse heights, and responsiveness to stimulation) indicate that it probably arises from action potentials generated by the surrounding neurons (Weber & Buchwald, 1965). In the previously mentioned habituation experiments (Kitzes & Buchwald, 1969) quantification of MUA responses was accomplished by a frequency analysis which weighted equally all spikes above a preselected amplitude. Each spike exceeding that amplitude triggered a pulse of constant size and duration. The resulting pulses were fed into an R-C integration circuit. The areas under the resulting integrated response profiles were proportional to the frequency of firing in the response. They were subjected to a multiple regression analysis in order to quantitatively assess response changes.

Stimulus-model comparator

Sokolov (1963) hypothesized that 1) responses in the afferent collaterals to the midbrain reticular formation (MRF) mediate arousal essential for orienting to initial presentations of a stimulus; and, 2) MRF afferent response decrements due to cortical inhibition underlie the process of habituation. Both statements lack direct confirmation. He based these proposals primarily on indirect evidence and upon the

assumption that EEG desynchronization adequately reflects reticular activation (Sokolov, 1960). He noted that cortical EP's can be recorded after behavioral and autonomic habituation appear to be asymptotic. He found decreases in stimulus intensity to be effective in reinstating the habituated response and the omission of a stimulus to be an "adequate stimulus" in OR evocation. These phenomena led him to assume that primary sensory transmission is unaffected in habituation. To account for introspective, behavioral, and autonomic indications that redundant stimulation can be effectively "ignored" he postulated reticular activation of the cortex as a necessary condition for OR occurrence. He hypothesized a stimulus-model comparator function at the cortical level, suggesting that when sensory input fails to match a "model of immediate experience" cortical inhibition of reticular neurons is released and orienting responses occur. When the stimulus does match the model, a function of repeated presentations, release of reticular inhibition ceases to occur.

Neurophysiological evidence for Sokolov's theory of habituation is scant. The hypothesis that MRF response decrements underlie habituation has received some support in EP, MUA, and single unit recordings. Hernandez-Peon (1960) reported decreases in amplitude of MRF evoked responses to repetitive auditory stimulation, but did not discuss their relationship to habituation. Recent data on MUA responses to repetitive auditory stimulation indicate response decrements coinciding with behavioral habituation (Saffran, 1969). This is consistent with reports

of single unit response decrements occurring as a function of stimulus repetition (Horn, 1966; Scheibel & Scheibel, 1968).

In addition to predicting MRF response decrements accompanying habituation Sokolov's model predicts that MRF activity initially augments primary sensory responses to a novel stimulus. Symmes and Anderson (1967) have found that MRF stimulation does result in an increase in amplitude of the EP late components in the inferior colliculus, medial geniculate, and auditory cortex. Lesions of the MRF have been found to interfere with orienting to auditory stimuli, indicating the importance of reticular activity in elaboration of the OR (Kesner and Vredenberg, 1970).

The lateral portion of the MRF receives collateral fiber input from secondary sensory fibers in the auditory pathway (Brodal, 1958; Whitfield, 1967). Single unit responses to auditory stimulation can be obtained from this area (Amassian & DeVito, 1954; Scheibel & Scheibel, 1968), as can MUA responses (Safran, 1969). It remains to be shown that lateral MRF responses reliably occur with orienting to initial presentations of a stimulus, or that they show consistent decrements with habituation to the stimulus.

Origins of inhibition

The assumption of either primary sensory afferent or MRF afferent response decrements underlying habituation leads to questions concerning the origins of the inhibitory processes mediating the decrements. Both Hernandez-Peon and Sokolov suggested possible inhibitory mechanisms.

Hernandez-Peon (1961) proposed that tonic inhibitory input from the MRF to the primary sensory nuclei increases with repetitive stimulation. He attempted to substantiate this hypothesis by showing that electrical stimulation of the MRF could reduce the amplitudes of subsequently evoked responses in the cochlear nucleus. Other experimenters have shown that midbrain tegmental stimulation effects on cochlear nuclear responses can be abolished by neuromuscular block (curare), or by section of the middle ear muscles (Hugelin, Dumont, & Paillas, 1960). Electrical stimulation of the MRF may fail to evoke the synchrony of discharges necessary to inhibit sensory afferent responses, making this kind of test of the hypothesis meaningless. A clear demonstration of an increase in MRF efferent activity to the primary auditory structures is required if the MRF is to be seriously considered the origin of hihibitory activity.

Direct cortical inhibition of cochlear nucleus, inferior colliculus, or medial geniculate responses is possible considering the existence of the necessary anatomical routes (Desmedt, 1960; Rassmussen, 1955; Zimmerman, Chambers, & Lui, 1964). Cortical efferents, described by Desmedt as being primarily inhibitory, originate in the temporoinsular cortex, descend along the lateral border of the reticular formation, and terminate in the cochlear nucleus. Evidence linking inhibitory output via cortical efferent projections to the primary auditory relays with observed instances of afferent inhibition is at present lacking (Saffran, 1969).

The habituation mechanism which Sokolov proposed depends upon cortical alteration of reticular activity. Possible anatomical substrates for this hypothesis have been identified. Pontine and MRF reticular units receive inputs from a number of cortical sites (Hernandez-Peon & Hagbarth, 1955; Scheibel, Scheibel, Mollica, & Moruzzi, 1955; Amassian & DeVito, 1954). Adey, Segundo, and Livingston (1956) found that responses to pontine reticular stimulation recorded in the centrum medianium could be blocked, augmented, or both by prior cortical stimulation. The areas which were found to be effective in altering brainstem conduction were the same as those from which French (1958) found stimulation to evoke MRF responses. Adey, et al. (1956) pointed out that those cortical areas which were most effective in blocking conduction were areas with extensive projections to the hippocampus, e.g., frontal orbital cortex, superior temporal lobe, anterior cingulate gyrus. While the studies mentioned above indicate the functional capacity of corticofugal activity to effect MRF responses and/or activity levels, an adequate demonstration of lateral MRF response decrements associated with increases in corticofugal activity is lacking. Similarly, parallels between these two kinds of response alterations and behavioral indices of habituation are not currently available.

The importance of hippocampal activity in evocation and habituation of the OR continues to be the object of much speculation. Observations of hippocampal slow wave activity associated with "arousal

reactions" first implicated hippocampal function in the process of orienting and subsequent habituation (Green & Arduini, 1954). The studies of Grastyan (1959) and Lissak & Grastyan (1960) indicated that the appearance of hippocampal theta activity (5-7Hz.) coincides with OR's to a novel stimulus and continues until OR habituation is complete. Grastyan suggested that hippocampal desynchronization represents a tonic inhibitory output to the MRF. This suggestion is consistent with the finding that hippocampal stimulation can block reticular conduction and that hippocampal output to the MRF is predominantly inhibitory (Adey, et al., 1956). They hypothesized that theta represents a decrease in that inhibitory output, effectively disinhibiting reticular activity and/or responses to the stimulus. The anatomical substrates for hippocampal influence on reticular activity have been shown to exist (Nauta, 1956). Reciprocal reticular - hippocampal - reticular connections provide a route by which input to the reticular formation could effect, and be effected by, hippocampal activity. Electrophysiological evidence for hippocampal projections to the MRF is also available (Green & Adey, 1965).

Parmeggiani's (1967) experimental analysis of reticular activity level on hippocampal activity led to the suggestion that a reticular - hippocampal feedback loop, via the septal area, underlies habituation. He found that moderate levels of reticular activation (produced by sciatic nerve stimulation) were accompanied by hippocampal theta activity, while stronger reticular stimulation produced hippocampal

desynchronization. This led to the hypothesis that stimulus evoked moderate levels of reticular activity result in hippocampal theta activity which represents a release in tonic inhibition of reticular activity. With continued stimulation evoked reticular activity could reach a level at which it would begin to elicit hippocampal desynchrony. The resulting inhibition of reticular responsiveness would then correspond to habituation.

In sum, the role of the hippocampal activity in effecting MRF responsiveness changes correlated with habituation is uncertain. The evidence that hippocampal input to the MRF is tonically inhibitory is fairly compelling (Adey, et al., 1956; Grastyan, 1959; Purpura, 1959). That reticular activity levels can, via the medial septal nucleus, differentially effect the degree of synchrony in hippocampal activity is also well established (Green & Arduini, 1954; Parmeggiana, 1967). Further the possibility of neocortical input exerting a modulatory influence on hippocampal activity has also been suggested (Adey, 1958). It remains to be shown, however, that any reticular response changes are consistently correlated with behavioral habituation or that hippocampal feedback to the MRF is responsible for response changes which do occur.

Dual-process theory

The habituation theories which have been discussed thus far might be considered neural systems models. Groves and Thompson (1970) have recently proposed a synaptic theory of habituation which may be

expanded into a system model as more data accumulate. Unable to find any habituation-correlated alterations in the afferents or efferents mediating the hindlimb flexion reflex in the acute spinal cat, they concluded that some interneuron process must be responsible for the reflex habituation. A single unit investigation of interneurons involved in the reflex revealed two types of response patterns. Some of the units (H) showed decreases in responses after repetitive stimulation. These decreases followed the time course of the reflex habituation. Other units (S) showed increased responding during the initial stimulus presentations, followed by decreased responding on successive stimulus presentations. The summation of these two response patterns was shown to correspond closely to the pattern of reflex habituation. Finding no interneurons that showed tonically increased responding with repetitive stimulation, they concluded that an extrinsic inhibitory process cannot account for the response properties of H and S units. This led to the hypothesis that the two populations of interneurons have intrinsically different response patterns and that they interact to produce habituation.

Two parallel, but interacting, systems for response elaboration and habituation were postulated. The S-R pathway represents "the most direct CNS route" from stimulus to response, while the "state" system includes pathways and structures, which determine the animal's level of responsiveness. The plastic interneurons in the S-R pathway they considered to be primarily H units, while the state system was

thought to contain most of the S units. They assumed both systems contained nonplastic units - units that do not vary in their responsiveness over successive stimulus repetitions. The course of habituation was proposed to be determined by interactions between the continuously decremental process within the S-R pathway and the transitory sensitization process within the state system.

An extension of this theoretical structure to a more general model for habituation was suggested. The authors proposed that the sensory input system "up to the level of the cerebral cortex" is composed of a series of nonplastic units. Similarly, the output system was assumed to consist of nonplastic units. In this theoretical framework habituation must be assumed to be a product of the intrinsic properties of cortical interneurons. The state system, in which Groves and Thompson included the reticular formation, hypothalamus, and limbic system, should interact with the S-R pathway to produce the typical course of habituation.

This expanded version of the dual-process theory has not yet been tested. Three key assumptions certainly require further investigation. First, they have assumed that no primary afferent response decrements occur subcortically. Second, they assert that there is no evidence for increased, presumably inhibitory, tonic activity as a function of repetitive stimulation. Third, their assumption that the state system contains predominantly S units would mean that some multiple unit, or single unit, responses within this

system should clearly show the sensitization effect.

It is evident from the data and theoretical notions reviewed there that important questions regarding the neural mechanisms underlying habituation remain unanswered. An evaluation of primary sensory and reticular afferent response changes coincident with habituation is needed. In order to evaluate the hypotheses of an active inhibitory mechanism operating to affect habituation, it is necessary to follow the time course of activity level changes in the neural sites that have been proposed as active inhibitors.

Methods

Introduction

In the experiment described here the course of cardiac OR habituation to a tone stimulus and to a stimulus change was followed. In the same animals multiple unit activity (MUA) responses to the original tone and to the stimulus change were recorded from the inferior colliculus (IC), medial geniculate (MG), auditory cortex (AC), lateral midbrain reticular formation (LMRF), and polysensory cortex (PSC) in rats immobilized with curare. Table 1 shows the neural recording sites for each animal. A frequency analysis of the MUA responses over successive five-trial blocks was used to determine whether or not any changes in the responses from these sites occurred as a function of stimulus repetition. The frequency analysis took into account all spike activity above a preselected amplitude surrounding the electrode and weighted spikes above that amplitude equally. This kind of analysis permitted evaluation of responses to the stimulus in terms of changes in frequency of neuronal firing.

The rats were immobilized with d-tubocurarine during the testing session. This was done to control for changes in neural response magnitude that might have been attributable to peripheral gating mechanisms; e.g., variations in middle ear muscle tonus or changes in animal orientation in the sound field. D-tubocurarine, given at the dosage levels used in this experiment, has been found to have little, if any, central nervous system effects (Skorobogatov, 1967).

Table 1

Neural Recording Sites for Individual Animals

	Electrode Locations							
Animal Number	IC	MG	AC	PSC	LMRF	MMRF	S	DHC
1						x	x	
3						x	x	
4							x	x
5							x	x
7						x		
7-I		x						
8								x
9								x
10			xx	x				
10-I		x						
11				x				
13				x				
16				x				
17			xx	x				
20-I	x				x			
21-I	x				x			
23-I	x				x			
24-I	x					x		
30-I		x			x			
31-I		x			x			
32-I	xx							x
34						x		x
35							x	
35-I		x				x		
37						x	x	

Cortical responses to sciatic nerve stimulation and EEG desynchronization resulting from reticular formation or thalamic stimulation were not affected by the administration of curare. Ochs (1959) found that previously reported effects of curare on direct cortical responses were probably attributable to vascular hypotension coincident with inadequate artificial respiration.

The purpose of this study was to determine the presence or absence of neural response changes correlated with OR habituation to repetitive stimulation. Consequently, tone stimuli of moderate intensity (60 db. above ambient noise level) which evoke cardiac OR's were used. The rate and number of stimulus presentations chosen were stimulus parameters for which cardiac OR habituation occurs.

As previously described, MUA can be observed by the use of amplifiers responsive only to impulses having rates above 500 Hz. Investigations of the relationship between this activity and the slower intracranial potential changes have indicated that MUA primarily represents axonal spikes (Schlag & Balvin, 1963; Weber & Buchwald, 1965), while evoked potentials and the gross electroencephalogram (EEG) contain a large contribution from summation of dendritic potentials (Purpura, 1958). MUA, while presumed to be primarily representative of a number of individual unit firings, can include summation of spikes from two or more concurrently firing units. Also, MUA evoked responses may include decreases as well as increases in individual unit activity. The MUA responses, therefore, represent

net neuronal firing elicited by the stimulus (Buchwald, et al., 1969). An evaluation of changes in this type of response during the time course of habituation was used in order to reveal neural unit firing characteristics which are altered as habituation is occurring.

In addition to studying MUA responses to the stimulus, the study described here involved recording MUA background activity from other neural sites. These sites, medial midbrain reticular formation (MMRF), septum (S), and dorsal hippocampus (DHC) are areas which might reasonably be considered to be involved in central mechanisms of habituation. It has been proposed that the moderate levels of MRF activity evoked by a novel stimulus should result in an increase in MUA in the septum (Parmeggiani, 1967). This increase could synchronize DHC activity. Synchronized DHC activity (theta) is said to represent a decrease in tonic inhibitory influence of the DHC on the MRF (Grastyan, 1959). With an increase in MRF activity, S activity level increases to a point at which it fails to synchronize HC activity, thereby reinstating tonic inhibition of the MRF. Background activity was recorded from these areas and a frequency analysis of high and low amplitude MUA was done in order to determine if the predicted activity level changes correlated with habituation of the OR.

Subjects

Twenty-five male albino rats (250-350 gm.) were used in the experiments. They were maintained under standard laboratory conditions and were water-deprived for 24 hours prior to surgery and testing.

Surgery

The rats were anesthetized with sodium pentobarbital (50 mg. Nembutal per kg. body weight) and bipolar stainless steel recording electrodes were stereotaxically implanted as described below. The neural electrode sites and their stereotaxic coordinates are given in Table 2. The electrodes had been prepared by use of the technique described in Appendix 1. The diameter of each electrode tip was approximately 100 μ, and the vertical tip separation between the two electrodes was approximately 0.4mm. Jewelers' screws were affixed to the rat's skull and the electrodes were lowered to the appropriate sites. One of the screws had an attached stainless steel wire to be used as an animal ground for the MUA recordings. The screws and electrodes were imbedded in dental acrylic and their leads soldered to a Winchester SRE - 7S subminiature connector. A stainless steel soldering flux was used to clean the leads prior to soldering, insuring good electrical contact to the connector. The connector and leads were then imbedded in dental acrylic. The skin and fascia were sutured, closing the incision around the electrode assembly.

The first sixteen of the rats were implanted with subcutaneous electrocardiographic (EKG) electrodes at the time of surgery. Their EKG electrodes consisted of stainless steel wire, the end of which formed a loop approximately 4 mm. in diameter. Except for the loop the wire was encased in teflon insulation. It was threaded subcutaneously from placements at the midline and lateral to the heart

Table 2

Stereotaxic Coordinates (Mm.) with Respect to Bregma

Location	Anterior	Posterior	Lateral	Depth
Medial Geniculate		5.9	2.9	4.7
Inferior Colliculus		8.1	2.2	3.4
Medial Midbrain Reticular Formation		6.3	1.0	5.3
Lateral Midbrain Reticular Formation		6.3	1.9	5.3
Dorsal Hippocampus		2.8	2.5	2.8
Medial Septum	1.0		2.5 (25°)	5.5
Auditory Cortex		4.0	7.0	.5
Polysensory Cortex	2.9		1.2	.5

to a point just posterior to the electrode assembly. In these animals the EKG electrode leads, as well as those from the intracranial electrodes, were soldered to the connector before it was imbedded in acrylic. Due to the acquisition of a third neural recording channel the remainder of the animals had needle EKG electrodes inserted subcutaneously at the time of recording with the leads being taken directly to an EEG machine instead of to the electrode assembly. For these rats, electrode placements were the same as for the chronically implanted animals with the exception that a third electrode, to be used as an EKG ground, was inserted at the base of the tail.

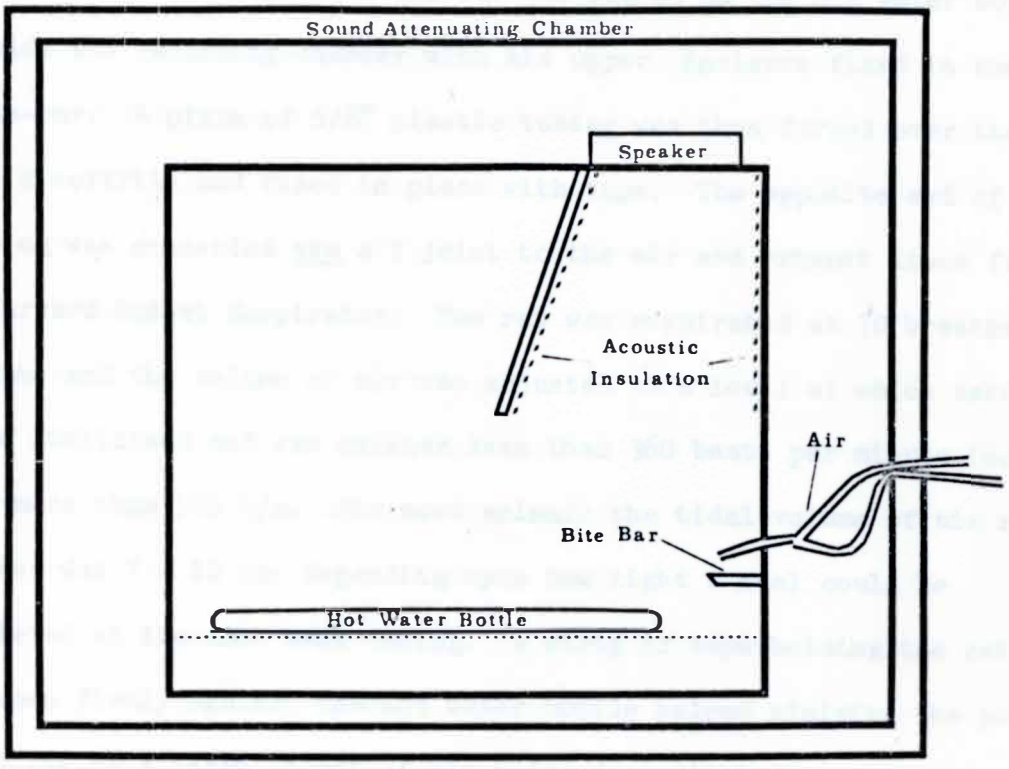
Testing apparatus

The testing apparatus consisted of a sound attenuating chamber (LVE) enclosing a Plexiglas box. The box was fitted with a bite-bar on one wall. With the rat's upper incisors secured in the bite-bar it could be fixed in an invariant position with respect to a speaker (Olson tweeter) mounted directly over the bar. Figure 1 shows a schematic representation of the testing apparatus. The walls surrounding the speaker, including the angled partition extending from the rim of the speaker to a point over the rat's head, were lined with sound absorbing material. This arrangement was designed to minimize sound wave reflection. There was an opening in the wall above the bite-bar for the respiration tube. The floor of the chamber consisted of a rubber hot water bottle.

Immobilization and respiration procedures

Approximately one week after electrode implantation the rats having

Figure 1. Schematic representation of the testing apparatus.



subcortical MUA recording electrodes were water deprived for 24 hours, then injected with .25 cc. d-tubocurarine. Because of the greater risk of electrode damage with a longer recovery period the rats having cortical electrodes were given a two day surgical recovery period. After the curare injection the rat was placed on the hot water bottle inside the recording chamber with his upper incisors fixed in the bite-bar. A piece of 5/8" plastic tubing was then forced over the rat's nostrils and fixed in place with tape. The opposite end of the tubing was connected *via* a Y joint to the air and exhaust lines from a Harvard Rodent Respirator. The rat was respirated at 70 breaths per minute and the volume of air was adjusted to a level at which cardiac rate stabilized and was neither less than 360 beats per minute (b/m) nor more than 550 b/m. For most animals the tidal volume of air required was 7 - 10 cc. depending upon how tight a seal could be achieved at the nose mask tubing. A strip of tape holding the rat's abdomen firmly against the hot water bottle helped minimize the possibility of a large amount of air being forced into the abdomen by the respirator.

Electrophysiological Procedures

The MUA electrode leads were connected *via* a matrix board to the amplifier inputs. This arrangement allowed the connection of any combination of electrode leads to any of the amplifiers. The amplifiers used for these recordings were designed with low noise (15μV.), high gain (2.5k X) characteristics. Their frequency response band was

500 - 10,000 Hz. A schematic diagram of the amplifier circuit is shown in Appendix 2. The MUA was recorded on magnetic tape using an Ampex 300 four-channel instrumentation recorder for some of the data and a SONY Quadradial four-channel tape recorder for the others. MUA was monitored on a Tektronix 565 Oscilloscope and 564 Storage Oscilloscope. Prior to recording, the MUA from each electrode site was checked for spike activity characterized by 1-2 msec. pulse widths and 30-150 μV amplitudes. Routine checks were made for switching transients and stimulus artifacts on the MUA recordings.

EKG recordings were taken from sixteen of the animals through an amplifier similar to the ones used for MUA recordings. The gain was 1000 X and the band pass, DC to 500 Hz. The schematic diagram for this amplifier is shown in Appendix 3. The EKG was recorded on one channel of the magnetic tape and monitored on the oscilloscope. For the remainder of the animals EKG leads were taken to a Nihon Khoden EEG machine where it was recorded on paper. All recordings were made inside an electrically shielded room.

Testing Procedures

After the rat was immobilized and satisfactory respiration was established the chamber was closed and the cardiac record observed for at least 10 minutes. If there were any signs of cardiac arrythmia the respiratory volume was readjusted and an additional check was made for cardiac stability. If the rat's cardiac rate was less than 360 b/m or exceeded 550 b/m tidal volume adjustments were made and

stability at an appropriate rate was established.

After the MUA had been checked for frequency and amplitude the testing sequence was begun. At random intervals averaging 90 sec. (60 - 120 sec.), timed by an LVE punched tape reader, a 5-sec. tone was given through the speaker above the rat's head. The tone, a sine wave train, was generated by a Wavetek signal generator. Its duration was determined by the B gate setting of the oscilloscope. The tone intensity was approximately 60 db above the ambient noise level in the testing chamber and was set by using a predetermined voltage level adjustment. The intensity measurements were made with a General Radio Sound Level Meter. Three pairs of tone frequencies were used in the experiment. Each rat received twenty tone trials of a frequency from one of the following pairs: 6k and 12k Hz, 2k and 10k Hz, 4k and 12k Hz. The stimulus change condition consisted of twenty trials of the other tone frequency of the pair. The output from the Wavetek was also taken to one channel of the tape recorder, and through a diode to the DC input of the EEG machine, providing a stimulus marker for the records.

Approximately 30 sec. before each stimulus presentation the tape recorder and EEG machine were switched to record and left running until after the tone presentation. After 20 stimulus presentations the tone frequency was changed and the intensity adjusted to a level that had previously been judged subjectively equal to the first tone. Twenty presentations of the second tone were then given. Finally, the tone

frequency was returned to its original setting and 5 trials were given.

After the final 5 trials the recording chamber was opened and the rat's rectal temperature measured. None of the rats for which data will be presented here showed temperatures more than 2°F below normal. The first 16 rats were subsequently allowed to recover from the immobilization. This was done to insure that the animals physiological condition during testing had not deteriorated to a level from which he could not recover. They were prefused the following day. Since the rats which were successfully respirated throughout the test sequence usually recovered completely from the immobilization, the remainder of the animals were perfused immediately following the testing.

Histology

The anode of a DC power source (3V battery) was connected to each electrode and the cathode to the cut edge of the animal's scalp for approximately 10 sec. Iron deposits from the exposed tip of the electrode reacting with the Prussian blue perfusant to form a bright blue marker were used to identify the tip location.

The rats were perfused first with 0.9% saline, then with the Prussian Blue perfusant described in Appendix 4.

The brain was then removed and fixed in 10% formalin for at least three days. The brains were frozen and sectioned in an AO cryostat microtome. The 30 μ sections were stained with cresyl violet and the

electrode locations microscopically verified.

Data Analysis

The cardiac data were recorded on chart paper at a speed of 6 cm./sec. and were scored manually. For each trial five pretone seconds and the five tone seconds were divided into one-second intervals. The length of the first five interbeat intervals in each of these one-second segments was measured to the nearest half millimeter. The measurement for each second was converted to rate in b/m based on average interbeat interval length (IBI). Rates in each tone second were averaged for five trial blocks, and rates for the 25 pretone samples in each five trial block were averaged. Standard errors (S.E.) were computed for the pretone means, and a pooled estimate of standard error was computed for the tone second means.

A frequency analysis of the MUA data was accomplished by the use of an RC "integration" network in which the output is proportional to the input activity frequency. Since an analysis of both negative and positive going potentials was desired the output from the magnetic tape was first taken through a rectifier. The rectified activity was then fed into a voltage comparator circuit. This circuit provided a single output for each input pulse above a selected amplitude voltage. The amplitude discriminator level could be set by movement of a potentiometer. The comparator output was fed into a DCL monostable multivibrator which provided an output pulse of constant voltage and duration for each input pulse. These uniform pulses were then applied

to an RC integration circuit with an adjustable fall-time constant (30 - 150 msec.). Appendix 5 shows a schematic diagram of the frequency analysis system. The output from the integration circuit was taken to a polygraph providing a graphic record of frequency changes before, during, and after each stimulus presentation.

The MUA from each site was analyzed at several discriminator levels and time constants. A combination which maximally displayed the responses to the stimulus was selected for each site and the data from that site recorded on chart paper.

Baseline activity prior to the tone onset was estimated for each trial and the area between the integrated output following stimulus onset and that baseline was measured. A K & E polar planimeter was used for these measurements. The response amplitudes thus obtained were averaged over blocks of five trials.

The MUA from the sites where a measure of background activity over successive trials was desired were subjected to frequency analysis at a low and a high discriminator level. Average output level of the integrator was measured for each prestimulus period and the resulting activity level measures averaged over blocks of five trials.

Results

Cardiac records for each trial were scored for mean IBI in each of 5 seconds prior to the tone onset (pretone sample), in each of the 5 seconds during the tone (tone sample), and in the first second following the tone offset. The trials were grouped in blocks of five trials each. A pretone mean rate and S.E. were computed for each trial block. The mean with a pooled estimate of S.E. for each of the tone seconds was compared with the pretone mean and S.E. for that trial block. A response was defined as any mean tone rate that was reliably different (S.E. limits falling outside the S.E. limits for the pretone samples, $p<.04$) from mean pretone rate. These cardiac responses to the tone were termed cardiac orienting responses (OR).

In 80 per cent of the rats, cardiac OR's to the first five tone stimuli in the 20 trial sequence were increases in average IBI following tone onset, or cardiac deceleratory responses. These rats will be called decelerators. Twenty per cent of the rats showed cardiac accelleratory responses, i.e., a decrease in mean IBI length, during one or more of the tone seconds. They will be referred to as accelerators. On the last trial block, Trials 16-20, of the Tone I sequence 90 per cent of the deceleratory cardiac OR's which had occurred on Trials 1-5 either did not occur or were decreased in amplitude and/or duration. This cessation of, or decrease in, responding in the last trial block was termed habituation.

Sixty per cent of the rats that showed acceleratory OR's partially

habituated to Tone I. Forty per cent of the animals showing initial accelerations did not habituate i.e., made OR's that were as large in amplitude and duration in the last trial block as they had been in the first trial block.

Seventy-nine per cent of the rats making deceleratory responses to the first tone also made deceleratory responses to the stimulus change condition, i.e., the first five trials of the Tone II sequence. The other 21 per cent of these rats did not respond on the initial trials of Tone II. In no case did rats making deceleratory responses to Tone I make acceleratory responses to Tone II.

Of the rats making acceleratory responses to Tone I, 80 per cent showed acceleratory OR's to the stimulus change condition, Tone II. The other 20 per cent made no significant acceleration or deceleration to Tone II. On Trials 16-20 of the Tone II sequence 89 per cent of the decelerator showed diminished responses or no responses at all. Eighty per cent of the accelerators showed diminished or no OR's.

When the stimulus frequency was changed back to Tone I, 70 per cent of the deceleratory again made deceleratory responses. The other decelerators made no significant responses. Sixty per cent of the accelerators made acceleratory responses when the tone was changed back to Tone I. The other accelerator made no significant responses.

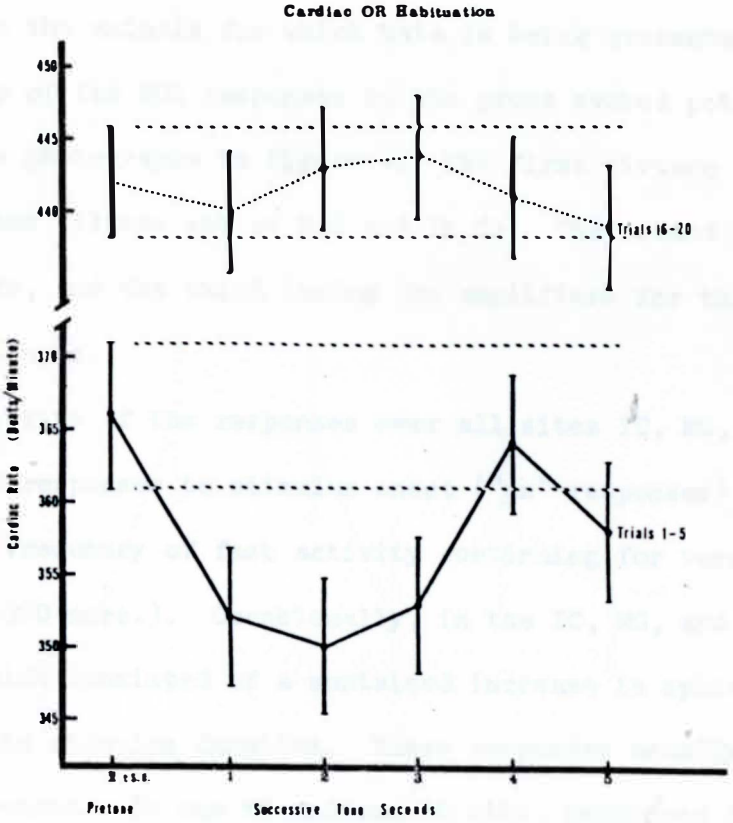
In Table 3 the percentages of decelerators and accelerators which completely habituated, partially habituated, and did not habituate are given. Figure 2 shows an example of a deceleratory cardiac

Table 3

Percentages of Rats Showing Cardiac OR Habituation

	Habituation		
Tone I			
	% None	% Partial	% Complete
Decelerators	10	20	70
Accelerators	40	60	0
Tone II			
Decelerators	11	11	78
Accelerators	20	20	60

Figure 2. Deceleratory cardiac response and its habituation. Cardiac rate during each tone-second (with a pooled estimate of S.E.) is shown with respect to mean (and S.E. limits) pretone rate.



response and its habituation. Figure 3 shows an example of an accelera-tory response on Trials 1-5 and partial habituation of the response on Trials 16-20.

The MUA was "integrated" as previously described and recorded on chart paper. The MUA responses evoked by the stimulus consisted of increases in spike activity. Instances in which the stimulus evoked decreases in frequency of MUA were noted in pilot animals, but were not found in the animals for which data is being presented here. The relationship of the MUA responses to the gross evoked potential is shown in the photographs in Figure 4. The first picture shows the EP with band pass filters set at 0.8 and 1k Hz. The second was taken at 80 and 10k Hz, and the third (using the amplifiers for this experiment) at 500 and 10k Hz.

The majority of the responses over all sites IC, MG, LMRF, AC, and PSC were responses to stimulus onset ("on" responses) with the increase in frequency of fast activity continuing for variable periods of time (50-300 msec.). Occasionally, in the IC, MG, and AC responses were seen which consisted of a sustained increase in spike frequency throughout the stimulus duration. These responses usually included an "on" component. In one MG and one AC site, responses to the stimulus termination "off" responses, were recorded. Figure 5 shows examples of response types.

The MUA response amplitude is proportional to the area between the level of integrated MUA during the prestimulus period and the level

Figure 3. Acceleratory cardiac response and its habituation. Cardiac rate during each tone-second (with a pooled estimate of S.E.) is shown with respect to mean (and S.E.) pretone rate.

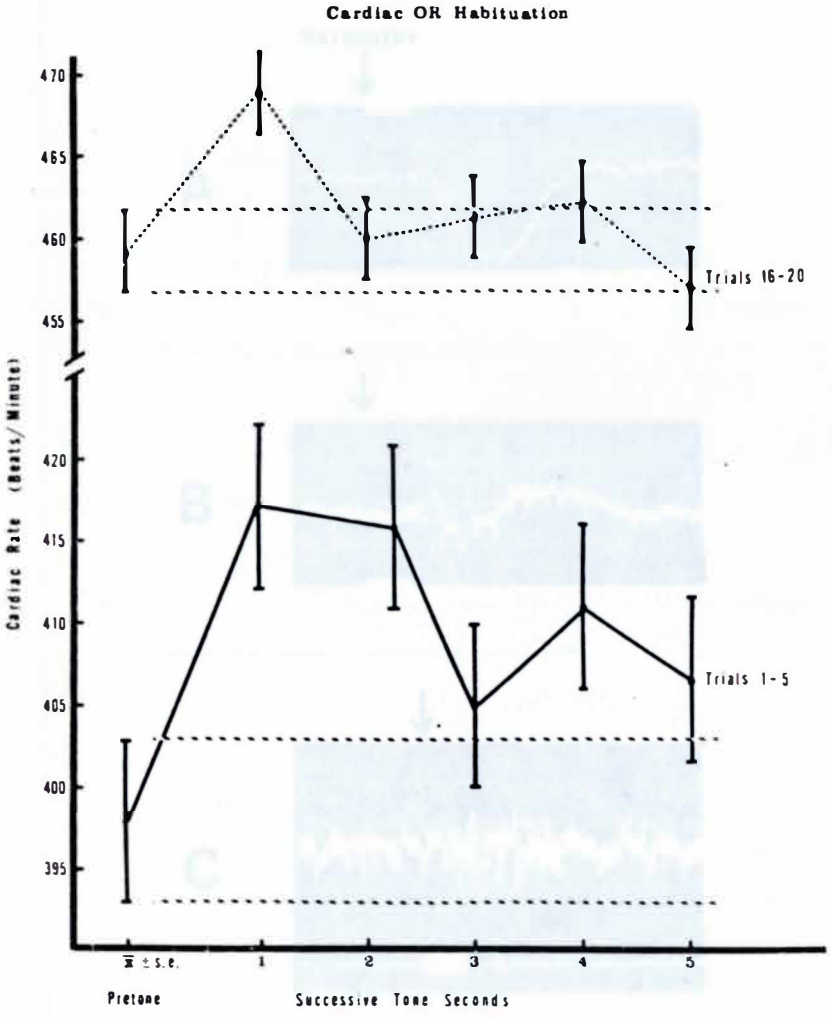


Figure 4. The relationship of MUA responses to the gross evoked potential (EP). Photograph A shows the evoked activity between 0.8 and 1k Hz; photograph B, between 80 and 10k Hz; and photograph C, between 500 and 10k Hz.

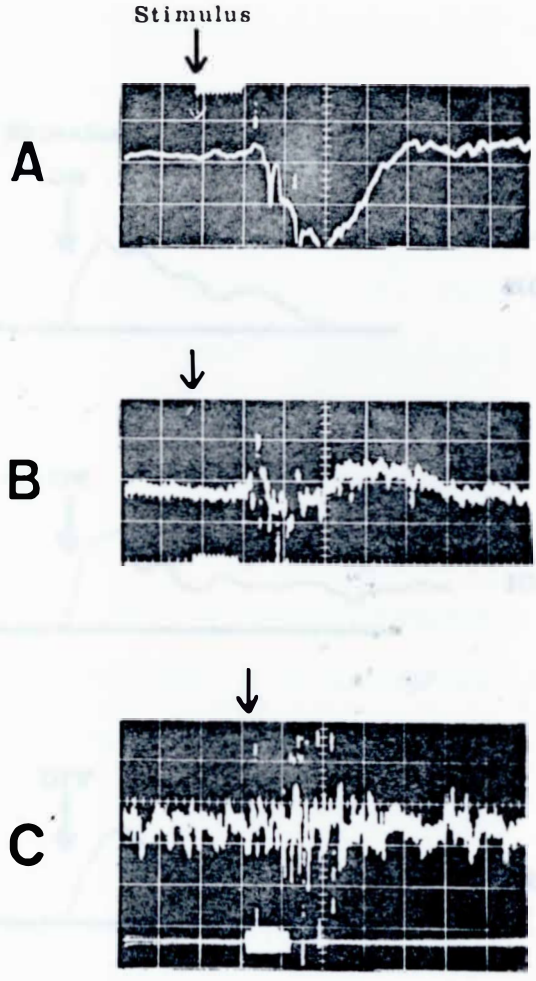
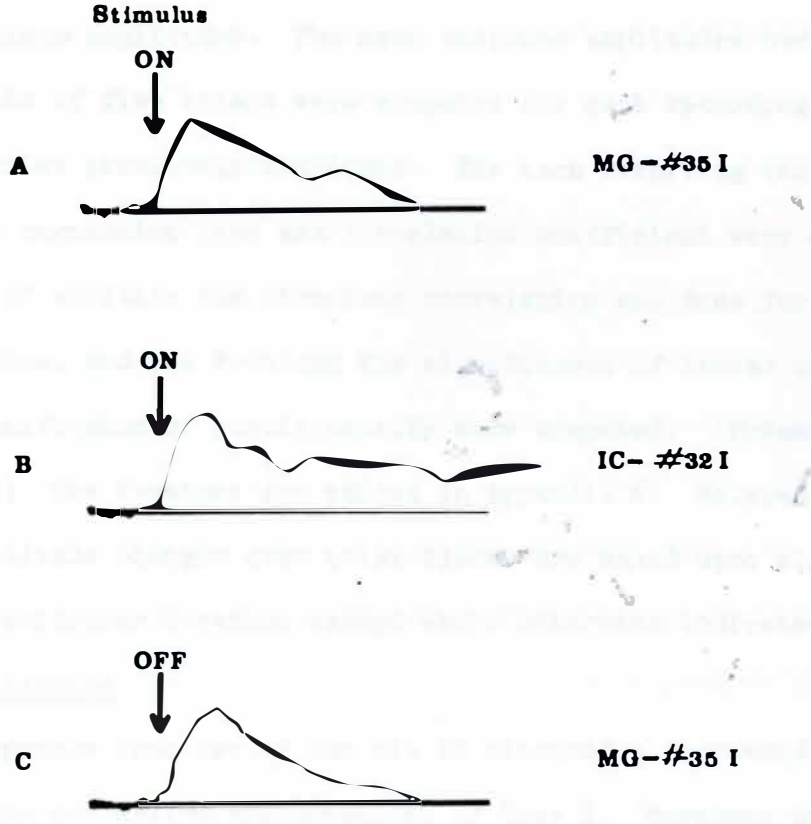


Figure 5. Types of MUA responses observed: A) An "On" response, B) A sustained response and C) "Off" response. The integrator output for these responses was recorded at a chart speed of 3 cm/sec.



following stimulus onset. Measurements of this area, over a time period determined by the nature of the response, were used as indices of response amplitude. In the case of discrete "on" responses, the area between the point of stimulus onset and the point at which the majority of the responses showed a return to baseline activity was measured. In the cases where the response was an "on" with gradually decreasing amplitude, the area measured was that between the stimulus onset and the point at which most response amplitudes declined to half of their maximum amplitudes. The mean response amplitudes over successive blocks of five trials were computed for each recording from the neural sites previously mentioned. For each recording the means and a linear regression line and correlation coefficient were computed. An analysis of variance for bivariate correlation was done for each regression line, and the F-values for significance of linear correlation and significance of curvilinearity were computed. (McNemar, 1962, p. 275) The F-values are tabled in Appendix 6. References to response amplitude changes over trial blocks are based upon significant linear or curvilinear F-ratios except where otherwise indicated.

Inferior colliculus

MUA responses from two of the six IC electrodes decreased in amplitude over successive presentations of Tone I. Response amplitudes from a third IC recording appeared to decrease, with the linear correlation value falling just outside the $p<.05$ level. Response amplitudes from two other recordings did not change significantly over

successive trial blocks. Finally, MUA responses from one IC recording increased over the first ten trials, then declined with subsequent stimulus presentations.

Tone II evoked MUA responses from only four of the six recording sites. In all four cases significant linear decrements in response amplitude occurred over successive blocks of trials. The responses to Tone II, Trials 1-5, were equal to or greater than the responses in the first block of Tone I trials.

Table 4 shows the IC MUA response changes for each recording and the course of cardiac OR habituation for the animal from which the recording was taken. As previously mentioned, cardiac OR habituation, whether partial or complete, usually occurred within the sequence of 20 tone presentations. MUA response decrements in the IC also occurred as a function of as few as 20 original stimulus presentations. Cardiac responses to the change in tone frequency (Tone II) habituated by the time the new tone had been repeated 20 times. Within this same time period MUA responses to the second tone decreased in amplitude.

It should be noted that in one case a MUA response decrement occurred when OR habituation was not complete. Also, one animal's MUA responses appeared to be decreasing when OR habituation was only partial. The MUA response which increased, then decreased, in amplitude was from an animal which did not habituate to the first tone.

In summary, during the period of time when OR habituation was occurring there are aggregates of cells in the IC that respond to

Table 4

Inferior Colliculus Response Changes as a Function of Cardiac OR Habituation

Animal*	Tone	Neural Responses			EKG Responses					
		Increase ↓ Decrease	Decrease	No Change	Accelerate	Decelerate	No Response	Habituation	Partial Habituation	No Habituation
32-I_L	I		x			x		x		
	II		x			x		x		
32-I_R	I		x			x		x		
	II		x			x		x		
24-I	I		x			x			x	
	II		x				x			
23-I	I			x		x		x		
20-I				x		x				x
21-I		x			x					
	II		x		x				x	

* L and R refer to left and right

initial presentations of an auditory stimulus, then show decreases in frequency of firing coincident with OR habituation. Second, there are units which also respond initially and show no changes in frequency of firing as a function of successive stimulus presentations. Finally, one group of cells from which recordings were taken responded to initial presentations of the stimulus then showed further increases in frequency of firing in a subsequent trial block. This process is sometimes called sensitization (Groves and Thompson, 1970). Figure 6 identifies the location of each IC electrode. The patterns of response change which were observed did not appear to be correlated with electrode location within the IC.

Medial geniculate

MUA responses from four of the six MG recording sites decreased in amplitude as a function of successive trials in the Tone I sequence. In three instances the response decrements were evident when the cardiac OR had only paritally habituated. Responses from two MG electrode locations did not change in amplitude as a function of successive stimulus presentations. Responses to the stimulus change condition, Tone II, decreased in amplitude in only two of the recordings. Responses from a third site appeared to decrease during the Tone II sequence; however, the linear correlation F-ratio was not significant at the $p<.05$ level. One MG response increased in amplitude over the first ten trials of Tone II, then decreased. Two MUA responses to Tone II did not change in amplitude during the 20 trial sequence.

Figure 6. Locations of IC electrodes. The number of the animal denotes the location of his IC electrodes.

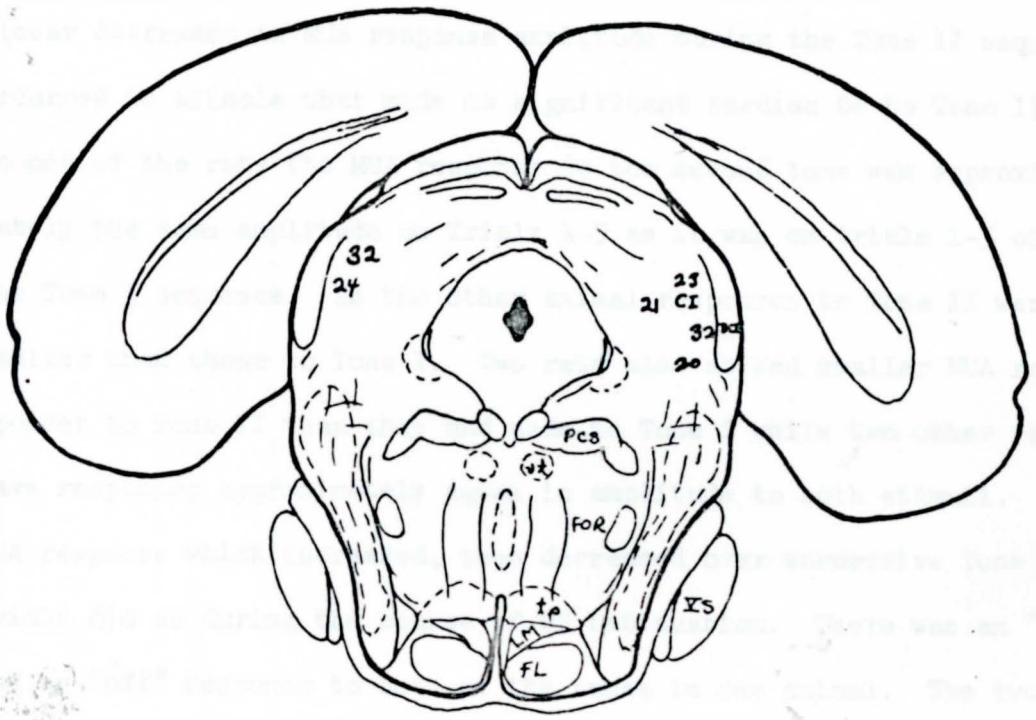


Table 5 shows the MG response patterns with the OR responses and response habituation for each MG recording. In addition to the three MUA recordings in which response amplitudes to Tone I decreased when cardiac OR's had not completely habituated, a fourth response showed a decrement which was coincident with OR habituation. Both of the linear decreases in MUA response amplitude during the Tone II sequence occurred in animals that made no significant cardiac OR to Tone II. In one of the rats the MUA response to the second tone was approximately the same amplitude on Trials 1-5 as it was on trials 1-5 of the Tone I sequence. In the other animal responses to Tone II were smaller than those to Tone I. Two rats also showed smaller MUA responses to Tone II than they had made to Tone I while two other rats gave responses approximately equal in amplitude to both stimuli. The MUA response which increased, then decreased over successive Tone II trials did so during the course of OR habituation. There was an "on" and an "off" response to both of the tones in one animal. The two responses exhibited the same changes as a function of stimulus repetition.

These results indicate that there are groups of units in the medial geniculate nucleus which respond to initial presentations of a tone stimulus, then exhibit response decrements with successive stimulus presentations. As with the IC response changes the MG response decrements occurred when OR habituation was incomplete. The MG cells showing an initial sensitization did so during the time course of OR

Table 5

Medial Geniculate Response Changes as a Function of Cardiac OR Habituation

Animal	Tone	Neural Responses			EKG Responses					
		Increase ↓ Decrease	Decrease	No Change	Accelerate	Decelerate	No Response	Habituation	Partial Habituation	No Habituation
31-I	I		x			x		x		
	II		x				x			
35-I	I		x		x				x	
("on")	II		x		x					x
35-I	I		x		x				x	
("off")	II			x	x					x
30-I	I		x		x				x	
	II	x			x			x		
10-I	I			x		x			x	
	II		x				x			
7-I	I			x		x			x	
	II			x		x		x		

habituation. There were also groups of cells which responded to the stimulus and showed no systematic change in frequency of firing over successive stimulus presentations. Figure 7 shows the locations of the MG electrodes.

Lateral medbrain reticular formation

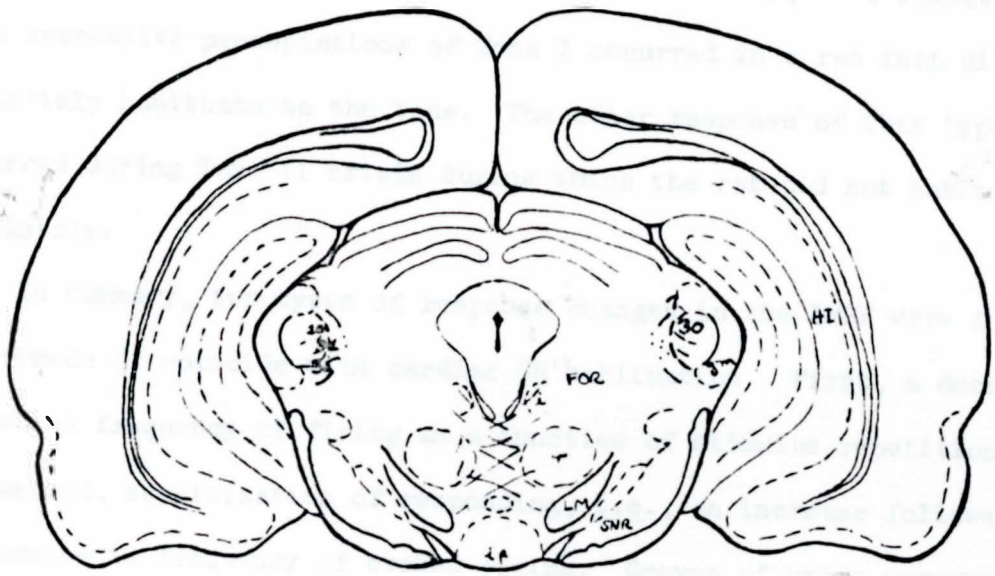
Lateral midbrain reticular formation MUA responses to the tones were more variable than those from the IC or MG. There were three instances in which linear response decrements appeared to occur, but due to greater variability within the trial blocks these changes failed to reach the significant level ($p<.05$) for linear correlation.

MUA responses from two of the six recording sites decreased in amplitude as a function of successive Tone I trials. Responses from two other electrode sites appeared to decrease over trials. The response amplitude from one LMRF site increased, then decreased during the first tone sequence (sensitization), and there was one LMRF response which showed no change in amplitude as a function of stimulus repetition.

MUA responses from two of the six recordings decreased significantly as a function of successive Tone II trials. A third response appeared to decrease but the degree of linear correlation between response amplitude and trials was not significant. Two responses showed no change in amplitude over trials and responses from one recording site increased then decreased during the Tone II sequence.

The LMRF responses to Tone II were equal to or greater than the responses to Tone I in four of the six recordings.

Figure 7. Locations of MG electrodes. The number of the animal denotes the location of his MG electrodes.



The cardiac OR habituation data for the rats from which these LMRF recordings were taken are presented in Table 6. Two of the response decrements in the Tone I sequence occurred in animals that did not completely habituate. One of the responses that increased in amplitude during initial presentations of a stimulus, then decreased over successive presentations of Tone I occurred in a rat that did not completely habituate to the tone. The other response of this type occurred during Tone II trials during which the rat did not habituate completely.

In summary, two types of response changes in the LMRF were found to precede or coincide with cardiac OR habituation. First, a decrease in evoked frequency of firing as a function of stimulus repetition, and second, sensitization of responding, i.e., an increase followed by a decrease in frequency of evoked firing. Groups of units responding to the stimulus without systematic changes over successive trials were also found.

The LMRF electrode locations are indicated in Figure 8.

Auditory cortex

Responses from the AC electrode sites exhibited few systematic changes as a function of successive stimulus presentations - either to Tone I or to Tone II. In Table 7 the neural and cardiac response data are presented. As with the LMRF sites response amplitude variability was higher than that for the IC and MG sites and some of the responses appeared to show systematic changes which did not prove to be signi-

Table 6

Lateral Midbrain Reticular Formation Response Changes as a Function of Cardiac OR Habituation

Animal	Tone	Neural Responses			EKG Responses					
		Increase ↓ Decrease	Decrease	No Change	Accelerate	Decelerate	No Response	Habituation	Partial Habituation	No Habituation
30-I	I		x		x				x	
	II			x	x			x		
21-I	I		x		x					x
	II	x			x				x	
31-I	I			x		x		x		
	II		x				x			
20-I	I	x				x				x
	II		x			x		x		
23-I	I			x		x		x		
	II			x		x		x		
25-I	I			x		x			x	
	II			x		x				x

Figure 8. Locations of LMRF electrodes. The number of the animal denotes the location of his LMRF electrodes.

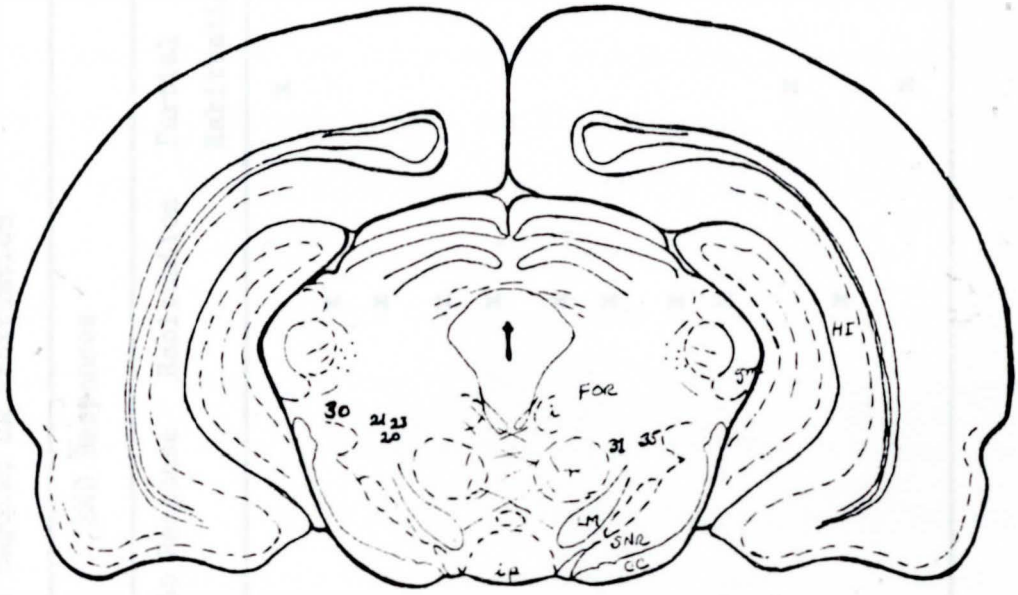


Table 7

Auditory Cortex Response Changes as a Function of Cardiac OR Habituation

Animal*	Tone	Neural Responses			EKG Responses					
		Increase ↓ Decrease	Decrease	No Change	Accelerate	Decelerate	No Response	Habituation	Partial Habituation	No Habituation
13	I		Xsc			x			x	
	II			x		x		x		
10_R	I			x		x		x		
("on")	II		Xsc			x		x		
10_R	I		Xsc**			x		x		
("off")	II			x		x		x		
10_L	I		Xsc**			x		x		
	II			x		x		x		
17_L	I			x		x		x		
	II			x		x			x	
17_R	I			x		x		x		
	II			x		x			x	

* L and R refer to left and right

** Marginally significant (.10>p<.05)

ficant at the $p<.05$ level.

One response decreased exponentially as a function of successive Tone I trials and two other responses showed marginally significant curvilinear decreases. One response to Tone II also showed a significent curvilinear decrease. The remainder of the responses to both the original stimulus and the stimulus change did not systematically change during the course of cardiac OR habituation. The MUA response that decreased significantly over Tone I trials did so in an animal that showed partial habituation to the tone.

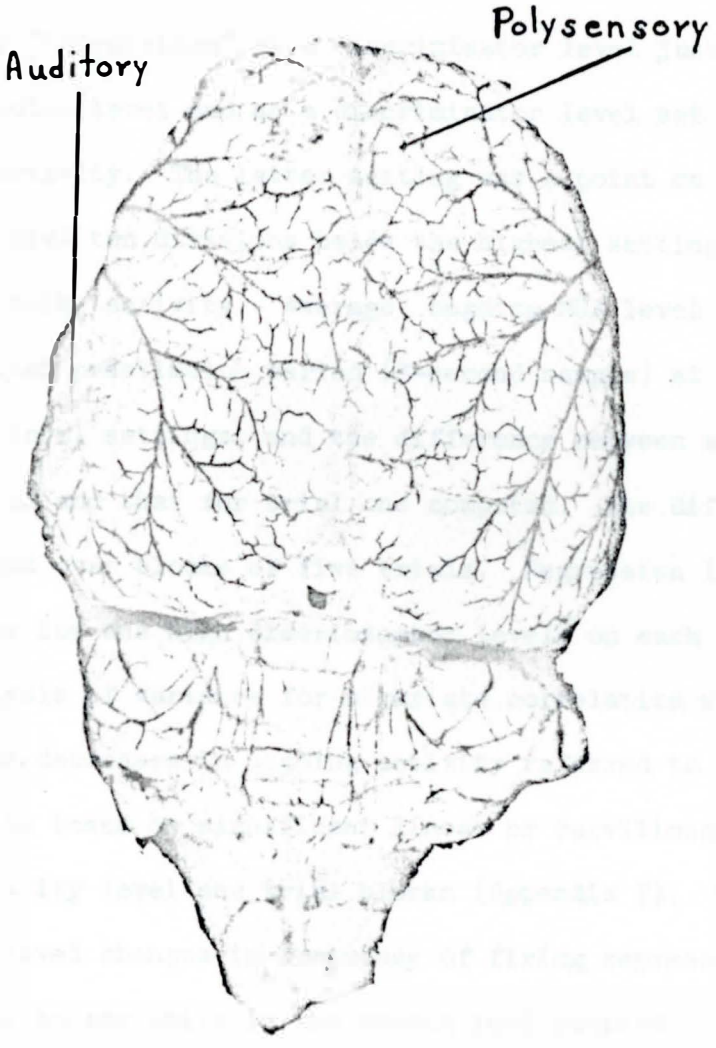
All of the AC responses to Tone II were equal to or greater than the initial responses to Tone I. Both and "on" and an "off" response was recorded from one auditory cortex electrode. The fact that the two responses underwent different systematic changes during the course of habituation suggested that the responses may have come from different cells in the pool of units surrounding the electrode.

Figure 9 shows the location of the AC electrodes.

Polysensory cortex

As with the AC responses, MUA responses from the area of the PSC exhibited few systematic changes in amplitude as a function of stimulus repetition. Only one of the six MUA responses showed a significant decrease in amplitude over the Tone I sequence. This occurred in a rat in which cardiac OR habituation occurred. One response to Tone II also decreased as a function of successive trials and during the course of cardiac OR habituation. Four of the five MUA initial responses to

Figure 9. Locations of AC and PSC electrodes are labeled.



Tone II were greater than or equal to initial responses to Tone I. (Table 8)

The location of the polysensory cortex electrodes is shown in Figure 9.

The septal, hippocampal, and medial midbrain reticular MUA was analyzed by "integration" at a discriminator level just above amplifier noise level and at a discriminator level set for high amplitude activity. The latter setting was a point on the discriminator dial ten divisions below the highest setting which revealed any spike activity. Average ongoing MUA level was determined for each prestimulus period (3-second sample) at both discriminator level settings, and the difference between activity level on each trial and that for trial one computed. The difference scores were averaged over blocks of five trials. Regression lines were computed for low and high discriminator levels on each tone sequence and an analysis of variance for bivariate correlation was done. The increases or decreases in ongoing activity referred to in the following pages will be based on significant linear or curvilinear correlations between activity level and trial blocks (Appendix 7). The low discriminator level changes in frequency of firing represent changes that could be due to any units in the neuron pool sampled. The high discriminator level data represent only high amplitude spiking. Table 9 gives the deductions that can be made from the relationship between overall and high amplitude MUA levels in the septal, dorsal hippocampal,

Table 8

Polysensory Cortex Responses as a Function of Cardiac OR Habituation

Animal	Tone	Neural Responses			EKG Responses					
		Increase ↓ Decrease	Decrease	No Change	Accelerate	Decelerate	No Response	Habituation	Partial Habituation	No Habituation
10	I			x		x		x		
	II			x		x		x		
13_L	I			x		x			x	
	II		x			x		x		
13_R	I			x		x			x	
	II			x		x		x		
11	I			x		x		x		
	II			x		x		x		
16	I		x			x		x		
	II									
17	I			x		x		x		
	II			x		x			x	

Table 9

Deductions That Can Be Made From The Relationship Between

Overall and High Amplitude MUA Level Changes

		High Amplitude MUA		
		Increase	Decrease	No Change
Overall MUA	Increase	High Amplitude Increase	Lower Amplitude Increase High Amplitude Decrease	Lower Amplitude Increase
	Decrease	High Amplitude Increase Lower Amplitude Decrease	High Amplitude Decrease	Lower Amplitude Decrease
	No Change	High Amplitude Increase Lower Amplitude Decrease	High Amplitude Decrease	No Change in High or Lower Amplitude

and medial midbrain reticular formation recordings.

Medial septal nucleus

High amplitude MUA in the S increased as a function of successive Tone I trial blocks in four of the six rats from which S recordings were taken. All of these animals made cardiac OR's to the tone, and all of them partially or completely habituated to the stimulus. Of the two rats that did not show increases in high amplitude S activity, one did not respond to the tone on Trials 1-5 but began responding later in the tone sequence, and the other did not habituate to Tone I.

During the Tone II sequence S high amplitude MUA in three of the five recordings decreased as a function of stimulus repetition. In one of the remaining animals there was an increase in S high amplitude MUA, and in the other there was no change. The three rats in which high amplitude MUA decreased either partially or completely habituated to the tone. The animal which showed an increase and the animal which showed no change in high amplitude MUA did not habituate to Tone II.

In Table 10 the MUA level changes for the S recordings and their relationship to cardiac OR habituation are presented. Figure 10 indicates the location of each S electrode.

Dorsal hippocampus

MUA levels in the DHC did not change consistently as a function of stimulus repetition, and the changes that did occur were not directly related to the degree of cardiac OR habituation. In Table 11 the DHC activity level changes are given in relation to cardiac OR habituation

Table 10

Medial Septal Nucleus MUA Changes as a Function of Cardiac OR Habituation

Animal Number	Tone I			Tone II		
	High Amplitude Changes	Lower Amplitude Changes	Cardiac OR	High Amplitude Changes	Lower Amplitude Changes	Cardiac OR
1	Increase	Decrease	Habituation	Decrease	Increase	No Response
37	Increase		Habituation	Increase		No Habituation
3	Increase		Partial Habituation	Decrease		Partial Habituation
5	Increase		Partial Habituation	---	---	---
35	Decrease		No Habituation	No Change		No Habituation
4	No Change	Increase	No Habituation	No Change	Increase	Habituation

Figure 10. Locations of S electrodes. The number of the animal denotes the location of his S electrodes.

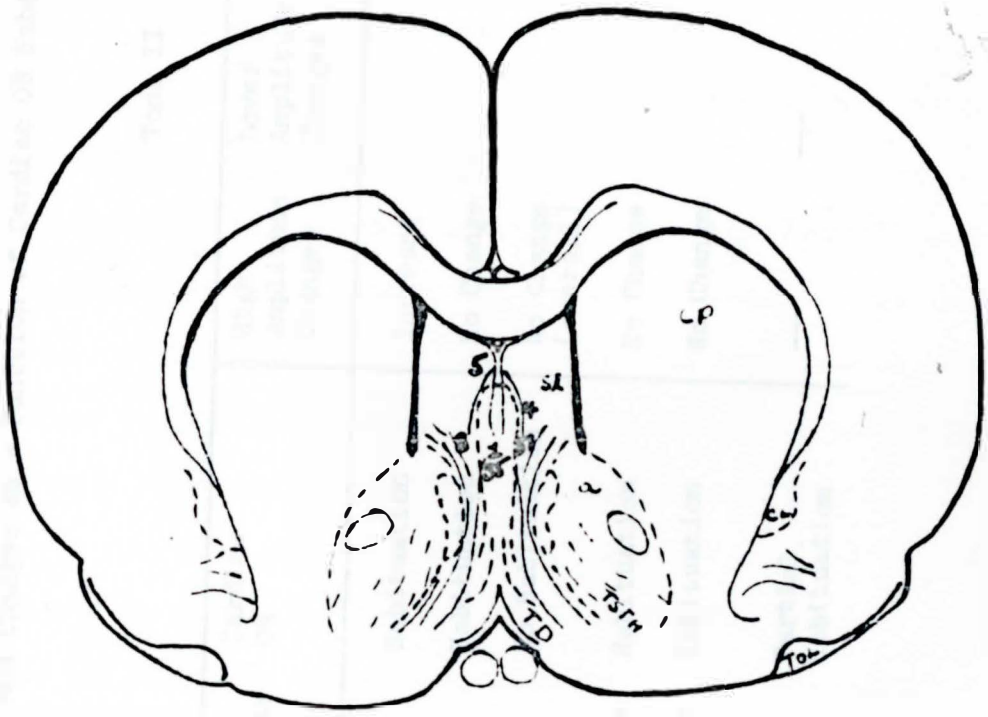


Table 11

Dorsal Hippocampus MUA Changes as a Function of Cardiac OR Habituation

Animal Number	Tone I			Tone II		
	High Amplitude Changes	Lower Amplitude Changes	Cardiac OR	High Amplitude Changes	Lower Amplitude Changes	Cardiac OR
32	Increase		Habituation	Increase		Habituation
34	Increase		Habituation	No Change		Habituation
4	Increase (overall)		No Habituation	No Change (overall)		Habituation
9	No Change	No Change	Habituation	No Change		Habituation
8	No Change	No Change	Habituation	No Change		Partial Habituation
5	Decrease	Increase	Partial Habituation	---	---	---

in the animal from which the DHC recording was made. As can be seen from the table, increases, decreases, and no changes in MUA level were found in animals that partially or completely habituated to the first tone. The Tone II data show that one instance of an increase and four instances of no change occurred in rats that partially or completely habituated to the tone.

In Figure 11 the locations of the DHC electrodes are indicated.

Medial midbrain reticular formation

In three of the six MMRF recordings high amplitude MUA decreased as a function of successive stimulus presentations. Of the three other MMRF recordings one showed an increase in high amplitude activity and the other two showed no change. All of these animals partially or completely habituated to the Tone I stimulus over trials.

The rats that partially or completely habituated to Tone II exhibited no changes in high or overall MUA during the Tone II sequence. One of the rats that showed a decrease in level of high amplitude MUA did not respond to the second tone. The other did not habituate. (Table 12)

The MMRF electrode locations are indicated in Figure 12.

Figure 11. Locations of DHC electrodes. The number of the animal denotes the location of his DHC electrodes.

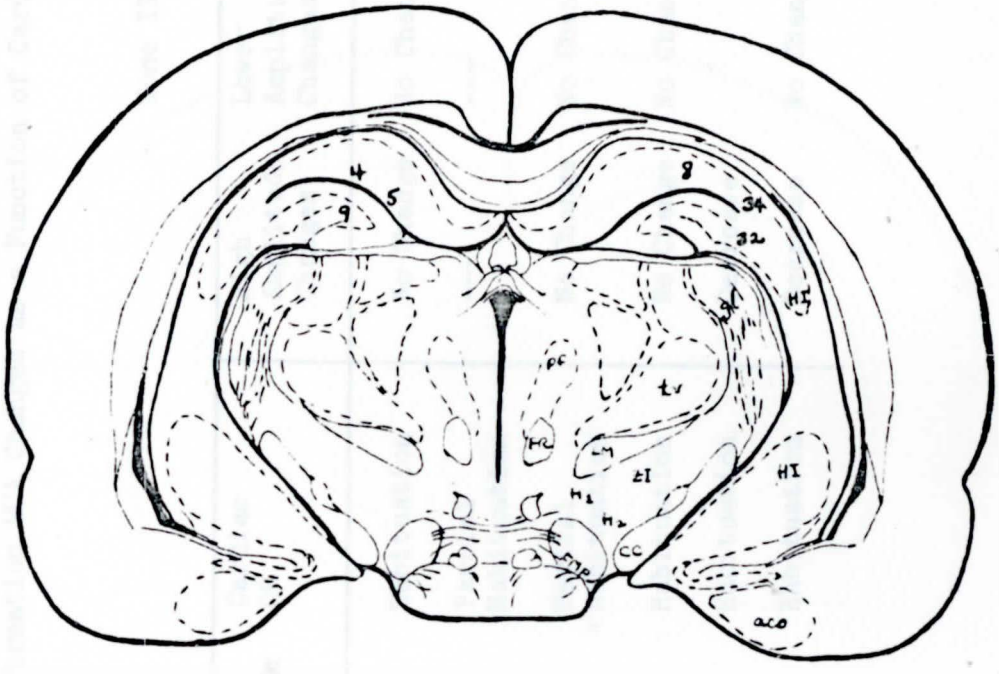
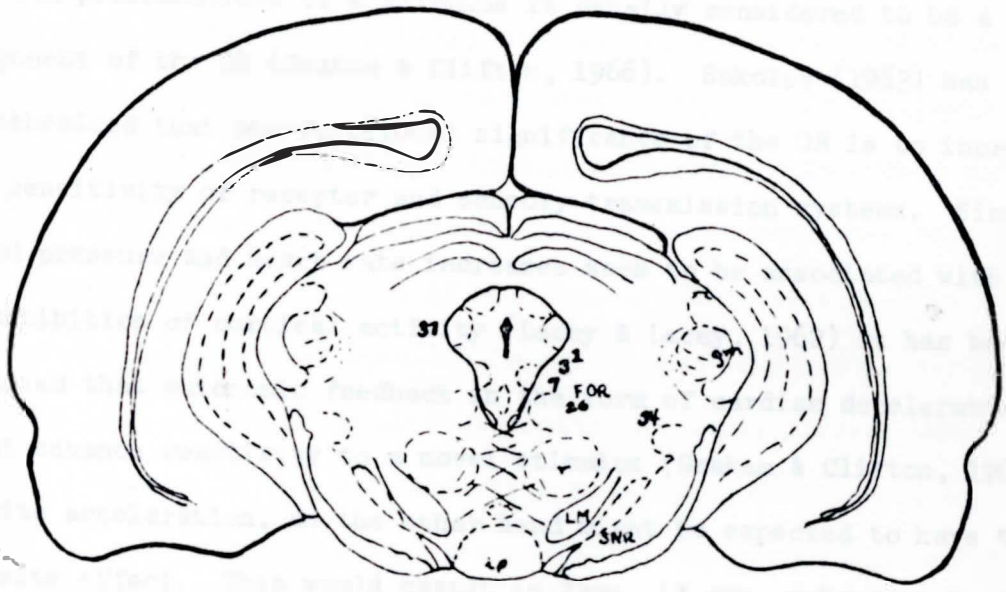


Table 12

Medial Midbrain Reticular Formation MUA Changes as a Function of Cardiac OR Habituation

Animal Number	Tone I			Tone II		
	High Amplitude Changes	Lower Amplitude Changes	Cardiac OR	High Amplitude Changes	Lower Amplitude Changes	Cardiac OR
34	Decrease		Habituation	No Change	No Change	Habituation
26	Decrease	Increase	Partial Habituation	---	---	No Response
3	Decrease		Partial Habituation	No Change	No Change	Partial Habituation
7	No Change	Increase	Habituation	No Change	No Change	Habituation
1	No Change	Increase	Habituation	Decrease		No Response
37	Increase	Decrease	Habituation	Decrease	No Change	No Habituation

Figure 12. Locations of MMRF electrodes. The number of the animal denotes the location of his DHC electrodes.



Discussion

The cardiac OR's found in the animals in the present study were primarily deceleratory responses. Some acceleratory OR's were also found; however, the response pattern for any given animal was consistently one or the other. The deceleratory cardiac response to initial presentations of a stimulus is usually considered to be a component of the OR (Graham & Clifton, 1966). Sokolov (1963) has hypothesized that the functional significance of the OR is to increase the sensitivity of receptor and sensory transmission systems. Since blood pressure and heart rate increases seem to be associated with an inhibition of cortical activity (Lacey & Lacey, 1962) it has been proposed that autonomic feedback in the form of cardiac deceleration might enhance reactivity to a novel stimulus (Graham & Clifton, 1966). Cardiac acceleration, on the other hand might be expected to have the opposite effect. This would result in less, if any, enhancement of reactivity as a function of the initial stimulus presentations (Graham & Clifton, 1966). According to these hypothesized relationships, habituation of the deceleratory OR should result in progressively decreasing responsiveness in sensory systems. With habituation of the acceleratory cardiac responses, and consequent release of inhibitory influences, sensory afferent responses, should increase (Graham & Clifton, 1966).

The cardiac data from this experiment does not support such a

direct relationship. However, it should be remembered that the MUA responses sampled do not necessarily represent an unbiased sample of all sensory responses. Of the five rats that made acceleratory responses only two showed MUA response sensitization, i.e., increases in response amplitude during the first 10 stimulus presentations. Further, MUA response sensitization occurred in one rat that made deceleratory responses. Finally, both accelerators and decelerators showed MUA response decrements over trials. Cardiac OR habituation was slower in the accelerators, lending some support to Graham and Clifton's (1966) assumption that the acceleratory responses represent "defensive" responses to what is for the animal an intense stimulus. Defensive responses have been defined as responses which either do not habituate at all or do so very slowly (Sokolov, 1963). The distinction between OR and defensive responses is therefore made on the basis of rate of habituation. Ratner (1970) has suggested that these different types of responses probably represent end points on a reactivity continuum. If this is the case, OR's (deceleratory) which habituate over the time period included in this experiment and defensive responses (acceleratory) which habituate more slowly might be thought of as representing an approximation of the midpoint on the continuum. If so, neither would be expected to exert an appreciable influence on sensory responsiveness in the CNS. Therefore, the response changes observed in the present experiment cannot be entirely attributed to an autonomic feedback mechanism.

The MUA responses recorded from the IC, MG, and AC electrode sites were similar to those reported by Kitzes & Buchwald (1969) for the cat and by Saffran (1969) for the rat. Most of the responses included an onset burst of MUA followed by a decrease in activity to the prestimulus level or slightly above that level within one second of the stimulus onset. This would seem to indicate that many of the units in these sites signal a change in impinging stimulation, while a few units provide information about the continuing presence or absence of a stimulus. Kitzes and Buchwald reported the presence of "off" responses in the IC as well as in the MG. In these recordings no IC "off" responses were found. There were, however, offset bursts in the MG and AC recordings. They also reported finding IC responses which consisted of sustained decreases in MUA. As was previously mentioned, some responses of this type were observed in IC's of pilot animals for this experiment, but there were none among the MUA responses included in the data presented here.

In several of the recordings reported here there was a MUA response to one of the tones, but not to the other. Each of the tone frequencies evoked a response in at least one animal; therefore, it did not appear that any particular tone frequency was an inadequate stimulus. It seems likely that MUA responses from the small neuron pools sampled are quite frequency specific. Further evidence that different units, or groups of units, contributed to responses evoked by each stimulus frequency comes from the fact that most of the

initial MUA responses to Tone I differed in amplitude and form from MUA responses from the same site to Tone II. Kitzes and Buchwald (1969) have reported that MUA responses from the MG and IC were extremely tone frequency specific.

The type of high frequency neural activity which was recorded in the present experiment has been characterized as consisting primarily of extracellularly recorded action potentials (Schlag & Balvin, 1963). The amplitude of the MUA may be influenced by the proximity of cells to the electrode tip, by the number of cells synchronously discharging, and by variations in the extracellular potential sizes. Buchwald and Grover (1969) have found areas of high cell density which showed lower amplitude MUA than areas with lower cell densities. They concluded that varying extracellular potential amplitudes must account for the amplitude differences. The evaluations of the increases and/or decreases in background activity levels during the course of habituation were made with the assumption that neither changes in the number of cell firings closest to the electrode nor increases, or decreases, in synchrony of discharge could account for alterations in high amplitude MUA. Although the latter possibility could not be completely ruled out, it seemed more likely that increases in high amplitude activity represents the recruitment of activity from large cells. Grover and Buchwald (1970) have shown correlations between cell size surrounding electrode locations and amplitude of MUA in the electrode's recording field.

Estimates of the number of units contirbuting to an MUA response from an electrode location are difficult to make. Individual unit discharges can be tentatively identified by spike height; however, this criterion does not take into account the presence of discharges having the same characteristic spike amplitudes. Also, large cell discharges more distant from the electrode may appear to be identical to small unit discharges closer to the electrode. Observations of the MUA from the sites sampled in this study usually resulted in estimates of fewer than ten different spike heights. For the reasons mentioned above this should be considered a conservative estimate of number of units sampled.

As previously mentioned, the MUA responses represent net change in unit firing in the area of the electrode. A change in response amplitude may reflect both excitatory and inhibitory effects on the cells sampled. The possibility exists that a suppression of firing in one group of cells might balance an increase in activity from another group. This kind of effect could obscure responses to a stimulus or minimize decreases in response strength coincident with habituation. In the latter case decreases in the high frequency firing burst of a response combined with decreases in amount of inhibition of firing comprising the response could result in the appearance of no change in response amplitude.

The integration circuit used in this experiment "counted" all spikes above a preselected amplitude. As a result spike heights

above this preselected point did not contribute differentially to the response amplitude. The changes in response amplitude represent only changes in the spike frequency comprising the response. If a response consists of one or two large amplitude units firing against a background of fast activity a change in their frequency of firing during the course of habituation might not appreciably affect the integrator output.

In this experiment IC, MG, and AC multiple unit responses to tone stimuli, and to a change in tone frequency, decreased in amplitude during the course of autonomic response habituation. A study (Kitzes & Buchwald, 1969) using 1-second stimuli, presented at rates of 1 per 5 seconds also revealed IC and MG MUA response decrements over a sequence of 100 stimulus presentations. The incidence of response decrements in their recordings was approximately the same for both structures. Saffran (1969) reported MUA response decrements in the IC and MG, but did not mention the relative proportions of decrements. In the latter experiment, response decrements were inferred from trial sampling procedures rather than quantitative measurement. Decreases in amplitude of the EP late component from IC (Webster, 1971) and MG (Wickelgren, 1968; Hall, 1968) have also been reported to occur with repetitive stimulation. Late components of the AC evoked potential have also been found to decrease with repetitive stimulation (Hall, 1968; Marsh & Worden, 1964; Wickelgren, 1968). Some indications of a decrease in the early component of the EP have been

reported (Cook, Ellinwood, & Wilson, 1968). Of these experiments only one represented an attempt, as did the present experiment, to relate observed response decrements to habituation. Saffran's (1969) data suggested that habituation preceded the neural response decrements. Weinberger, et al. (1969) have also suggested that auditory evoked potential changes are not necessarily related to habituation. They failed to find lateral lemniscus EP decrements related to sound-elicited eye movement habituation.

In no case did the MUA response amplitudes drop to zero by the twentieth, and last, trial in a sequence. The IC and AC responses evoked by the tone did not decrease significantly in as many cases as did the MG responses.

The data from the present experiment indicate that response decrements in the IC, MG, and AC do occur during the time course of habituation. More important, some of the observed decrements occurred before complete habituation of the cardiac OR had taken place. In addition, there are groups of units in all three areas which do not show changes in response amplitude. It should be noted that the responses which decreased in amplitude did not disappear entirely. In light of these findings the idea that habituation cannot involve primary sensory response decrements while functioning as an adaptive behavioral change (Worden, 1966) is seriously questioned. Information about the stimulus is being transmitted after the OR has habituated. The fact that a change in tone frequency can be detected after responses

to the original tone have diminished indicates that a considerable amount of stimulus information is still available. The data presented here include a large number of responses to stimulus change which were larger than the response to the original stimulus. The fact that the stimulus change evoked cardiac OR's in most of the rats also indicates that a considerable amount of stimulus information is available to the animal after habituation has occurred.

IC and MG responses which increase in amplitude as a function of initial stimulus presentations have not previously been discussed. One of the IC responses in an MUA study (Kitzes & Buchwald, 1969) appeared to increase over successive trials. The significance of the increase cannot be determined because they did not test the regression lines for curvilinearity. A recent study of human auditory evoked responses showed enhancement of responses as a function of increased "attention." There are also reports of increases in visual cortex EP late components as a function of stimulus repetition (Kimura, 1962; Schwartzbaum, 1971). There are also indications of EP enhancement as a function of "attending" to the stimulus (Ohman & Lader, 1972). The possible relationship between the apparent sensitization of initial responses to the repetitive stimulus and other evidence for "sensitization" units within the CNS (Groves & Thompson, 1970; Tegler, Roemer, & Thompson, 1972) will be discussed in a later section of this paper.

There is little previous MUA data with which to compare the LMRF

and PSC response changes found in this experiment. Saffran (1969) reported that MUA responses in the LMRF decreased over successive stimulus presentations. She did not quantify the decrements, but did mention that they coincided with habituation of a behavioral OR. Single unit studies have identified LMRF units which show decreased responding after repetitive stimulation (Horn, 1966; Scheibel & Scheibel, 1968). The initial increases in MUA responses from the LMRF have not previously been reported; however units with this characteristic have been found in cat spinal interneurons (Groves & Thompson, 1970) and in descending pyramidal units (Tegler, et al., 1972).

Polysensory cortex EP responses in the cat have been found to decrease during orienting to a novel stimulus and increase as behavioral orienting habituates (Thompson & Shaw, 1965). This was not observed in the present MUA recordings from the rat's polysensory cortex. The MUA responses were, however, small and variable as would be predicted from Thompson and Shaw's data.

It seems unlikely that any of the MUA response amplitude changes found in the present experiment can be attributed to peripheral gating factors. The animals were curarized and maintained in a fixed position with respect to the stimulus source. It is also unlikely that changing physiological condition of the animal contributed to the response changes. The animals were respirated with a volume of air adequate to maintain normal cardiac activity throughout the recording session and

body temperature was maintained at normal levels. Cardiac and MUA response recovery in the stimulus change condition of the experiment, plus a five-trial check for responsiveness to the original stimulus at the end of the session further justify the assumption that the response changes were not a function of deteriorating physiological condition. Finally, the first 16 rats in the experiment were allowed to recover from the immobilization. Recovery time ranged from 30-90 minutes after the end of the recording session and recovered animals appeared healthy.

Measurements of MUA from S, DHC, and MMRF electrode sites were made in order to test the hypothesized S-HC-MMRF interrelationships. According to Parmeggiani (1967) septal activity should show progressive increases in MUA during orienting and habituation. DHC activity should decrease with orienting to the novel stimulus, then increase as habituation progresses. MRF activity should increase over initial stimulus presentations, then decrease.

In the medial septal nucleus there were consistent high amplitude MUA increases related to habituation to Tone I. This activity level change is consistent with Parmeggiani's hypothesis (Parmeggiani, 1967). The septal activity observed during the Tone II sequence was highly variable. Because of this variability and the small number of animals, the significance of the observed changes in activity is problematical. These results present an additional problem in interpretation as there are no referents in the literature to septal MUA level changes as a

function of habituation.

The activity level increases found to occur in the Tone I sequence have some implications for the previously mentioned theory of habituation mechanisms in that they could possibly represent an increase in septal output to the DHC. The increase in septal MUA were the only increases in activity level found to be consistently correlated with habituation to Tone I. Because of the possibility that these increases could represent an increase in modulatory output from the septal area, it would seem that further exploration of the relationship between septal function and habituation might prove profitable. There are indications that the septal area is involved in some aspects of habituation, because medial septal lesions have been found to result in slower habituation to the kind of tone stimuli used here (Sanwald, et al., 1969).

In the DHC and MMRF recordings, no pattern of MUA level change was consistently correlated with habituation or the lack of habituation. It is possible that the MUA recordings from the small cell populations in the DHC are not sufficiently sensitive to reflect overall changes in output. Alternatively it would seem that HC output may at best be secondarily related to habituation. In any case while not disproving the septal-hippocampal-reticular circuit that Parmeggiani (1967) proposed, there is little evidence from the data to support the hypothesis.

The findings in this study and the results of previous experiments

cast considerable doubt on the validity of the primary afferent inhibition hypothesis in its simplest form. The afferent inhibition concept as Hernandez-Peon (1961) formulated it assumes that a general inhibitory influence on the primary sensory relays functions to decrease response amplitude as habituation progresses. The fact that all groups of IC and MG units sampled in the present study did not show response decrements, and the finding that several cell groups showed an initial sensitization suggest that an overall decrease in responsiveness does not occur during habituation. Further, the hypothesis that MMRF output exerts the requisite inhibitory effect was not supported. Hernandez-Peon's (1961) original hypothesis of MMRF inhibition acting on primary sensory afferents was based upon data from stimulation experiments. These data have been shown to be influenced by the effects of middle ear muscle contractions (Hugelin, et al., 1960).

In the present experiment there were no consistent increases in MMRF activity during the time course of habituation. This finding casts further doubt on the proposed reticular origin of inhibition. The MMRF electrodes did not, of course, sample every point from which inhibition might originate. However, there did not appear to be any correlation between MUA level changes and electrode placement in the MMRF.

Other possible sources of primary afferent inhibitory effects, such as the corticofugal fibers described by Desmedt (1960) and

Rasmussen (1955) were not investigated in these experiments. Because of the kinds of response changes noted in the IC and MG, corticofugal influences on these sites would have to be of two qualitatively different sorts. Some facilitating influences must be postulated to account for the sensitization effects, and some inhibitory influences postulated to account for the response decrements. The same sort of corticofugal influences could produce the effects observed in the LMRF.

The design of the present experiment did not allow a direct test of this hypothesis because MUA recordings from the IC, MG, and LMRF could not be shown to be from the corticofugal fiber groups terminating in these structures. Indeed the patterns of observed responses in these areas could not have been predicted a priori.

The stimulus-model comparator mechanism of habituation which Sokolov (1961) proposed makes several assumptions that were not substantiated in this experiment. First, he proposed that subcortical primary sensory responses to a stimulus are invariant during the course of habituation. This was clearly not the case. Second, he proposed that reticular response augmentation of evoked responses at the cortex is necessary for orienting and that habituation involves cortical inhibition of these reticular responses. The present results do not support this hypothesis in that reticular response decrements were not consistently correlated with habituation as required by the hypothesis. Further, fewer response decrements were observed in the auditory cortex than in the other sensory afferents, making the failure

of augmentation idea even less credible. There is, however, one indication that this hypothesis might have some merit. The auditory cortex response decrements which did occur were exponential decays in amplitude. Amplitude decreases of this type suggest a possible early response augmentation.

As can be seen from the previous discussion the MUA response and activity level changes which were found in this experiment do not consistently support any of the theories postulating extrinsic habituation mechanisms. However, the increase in septal activity, potentially reflecting active inhibition, allows for the possibility that extrinsic synaptic mechanisms effect habituation.

The most recent hypothesis concerning habituation mechanisms is one which proposes that habituation is a function of intrinsic neural mechanisms. (Groves & Thompson, 1970). According to this formulation the intrinsic properties of individual interneurons are thought to interact to effect habituation. Groves and Thompson (1970) have found different classes of these interneurons involved in habituation of the cat hind-limb flexion reflex. One class of units, "H" units, show response decrements as a function of habituation. Another class of units, "S" units, show an initial sensitization, followed by response decrements. The type of response changes observed here in the IC, MG, Auditory Cortex, LMRF, and Polysensory Cortex are consistent with those reported by Groves and Thompson for spinal interneurons and Tegler, et al. (1972) for pyramidal fibers. Groups of units showing

response decrements ("H" units), units showing sensitization ("S" units) and apparently nonplastic groups of units were identified. The dual-process theory would predict more "S" units in the LMRF. While more "S" units were found there in these experiments, the small sample size limits the generality of the findings. The presence of "S" response patterns to the stimulus change as well as to the original stimulus (Tone I) also agrees with Groves and Thompson's (1970) data. As with spinal interneuron response patterns the "H" and "S" MUA response changes were found to be correlated with habituation. Just how these "H" units "habituate", or the "S" units become sensitized, is a difficult question to answer. Groves and Thompson (1970, p. 436) have suggested that the mechanism "...could include a variety of biochemical processes having relatively long time constants, glial cell actions, and as yet unknown mechanisms..." The hypothesis of a process other than an active inhibitory one must presuppose the existence of auditory "H" and "S" units for a variety of tone frequencies, since response recovery occurs with changes in tone frequency. The present data indicate that the response patterns of a group of cells can, in fact, differ as a function of stimulus frequency. More extensive investigations of cell population responses during the course of habituation are required before the interactions between "H", "S" and "NP" units can be specified.

Summary

The results of this experiment provide clear evidence of MUA auditory response changes as a function of habituation. Response decrements were found in the primary auditory system and in the MRF. In a number of cases the decrements were evident when cardiac OR habituation was incomplete. This suggests that sensory response decrements are concurrent with, and possibly precede OR habituation. MUA responses from both the primary and reticular sensory systems showed sensitization during the first half of the habituation sequence. These response changes were also coincident with, or preceding, OR habituation. MUA responses that did not change in amplitude during the habituation trials were also found. In the absence of any evidence strongly supporting either the primary afferent inhibition hypothesis or the stimulus-model comparator hypothesis it was concluded that the data are most consistent with Groves and Thompson's dual-process theory of habituation.

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Appendix 1

Electropolishing technique

The electropolishing technique used to prepare electrodes for this experiment was one commonly used in the preparation of small diameter metal specimens for field ion microscopy (Mueller, 1960). The basic principle involved in the procedure is as follows. When a fine wire completes the electrical circuit in an etching bath the wire surface begins to etch at a rate proportional to the current density gradient along the wire surface. The current density is proportional to the radii of curvature of points along the wire surface. The perimeter of the cut edge of the wire, having the greatest radius of curvature, etches more quickly than the remainder of the wire surface producing a tapered tip. Due to the interaction between current density as a function of radius of curvature and current density as a function of the downward flow of ions along the wire surface (viscous boundary layer) the current density gradient along the wire surface stabilizes. The wire continues to etch maintaining the tapered "end-form". The degree of taper of the "end-form" is determined by the circuit voltage. This relationship is due to differential rates of diffusion of the reaction products forming the viscous boundary layer. At high voltages the reaction products form a relatively thick boundary layer resulting in less etching along the horizontal dimension of the wire. The result is an end-form that is less tapered than that which occurs with a lower etching current. By empirically determining the appropriate etching-current level, electrodes of any tip

diameter and degree of taper can be made.

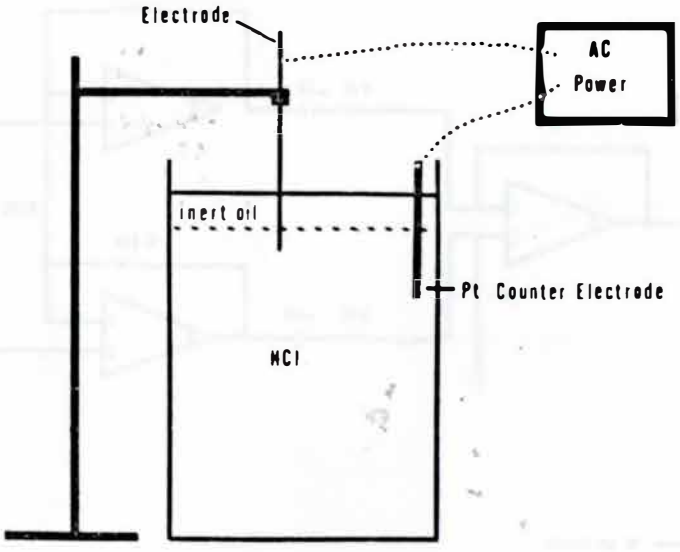
The material used for the electrodes in this experiment was #300 series stainless steel wire (.008" diameter) and the etching bath was 10% HCl. A platinum foil counter-electrode was used to complete the circuit in the etching bath. Four to 5 volts AC was applied between the wire and the bath. Figure 13 shows how the electropolishing procedure was implemented. The mineral oil layer was used to prevent spattering of HCl as etching proceded to the surface of the electrolyte. The electrode wire was lowered $\sim$ 3 mm below the HCl surface and the current applied until the wire had etched back to the acid-mineral oil interface (usually $\sim$ 1 min.).

After electrodes of the desired taper and tip diameter had been prepared they were cleaned with butanol, 20% HCl (hot), and rinsed with deionized water. The butanol was used to remove any remaining mineral oil film and the hot HCl to remove any oxide film which had accumulated on the etched surface. The electrodes were then coated with epoxylite (2 parts Epoxylite-1 part solvent) and baked at 140°F for 30 minutes. Second and third coats of epoxylite were subsequently applied and baked.

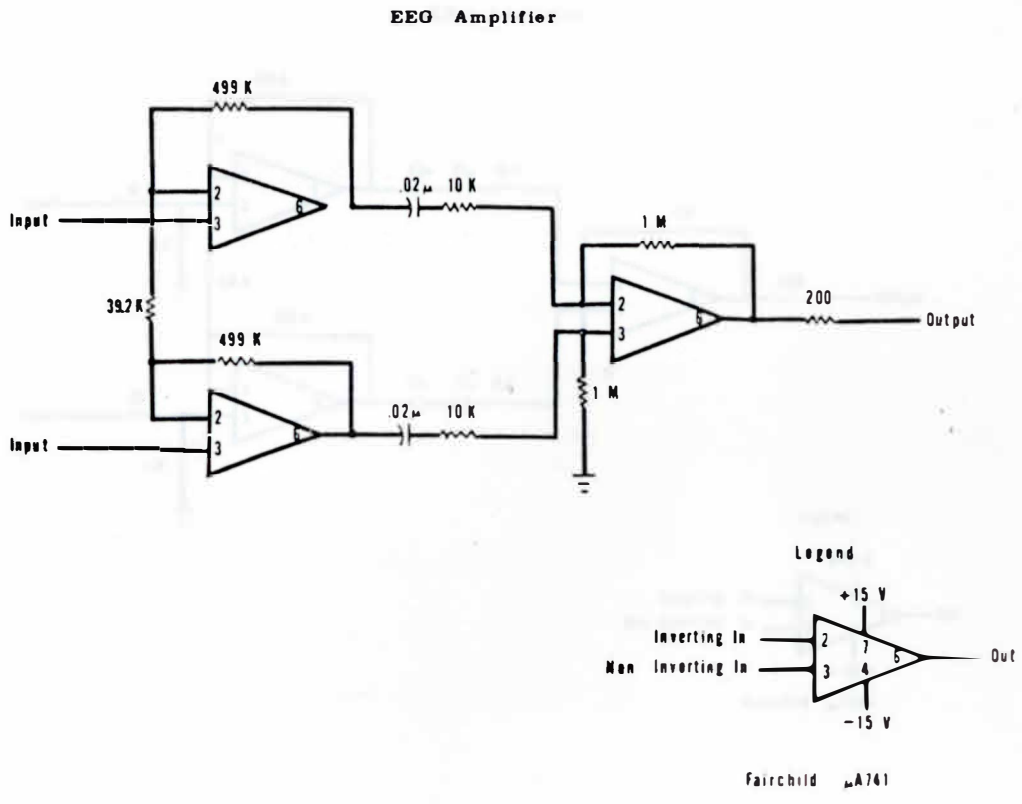
Finally, the area of the uninsulated tip of each electrode was confirmed using the "bubble test" described by Bures, et al. (Bures, Petran, & Zachar, 1962, pp. 160-161). If the exposed area was too small the tip was placed under a dissecting microscope and a #11 scalpel blade was used to scrape away excess insulation.

The electrodes were paired and the two cemented together with a vertical tip separation of $\sim$.4 mm.

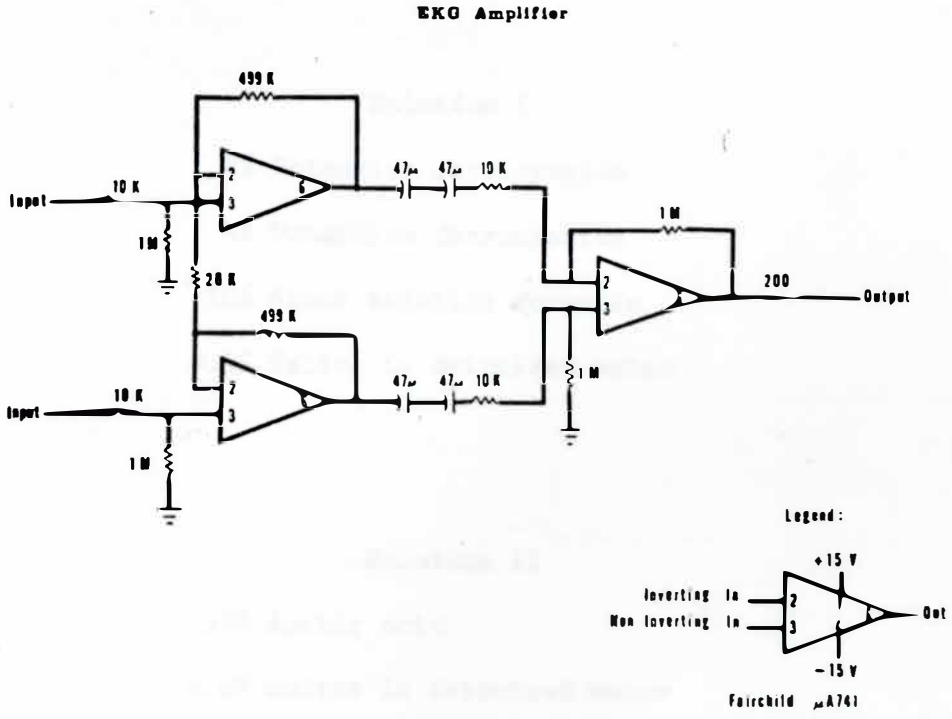
Figure 13. Electropolishing apparatus.



Appendix 2. Schematic Diagram of EEG Amplifiers



Appendix 3. Schematic Diagram of EKG Amplifiers



Appendix 4

Prussian Blue Perfusant

Equal parts of the following two solutions were mixed just prior to use:

Solution I

4% Potassium ferricyanide

4% Potassium ferrocyanide

10% Stock solution formalin

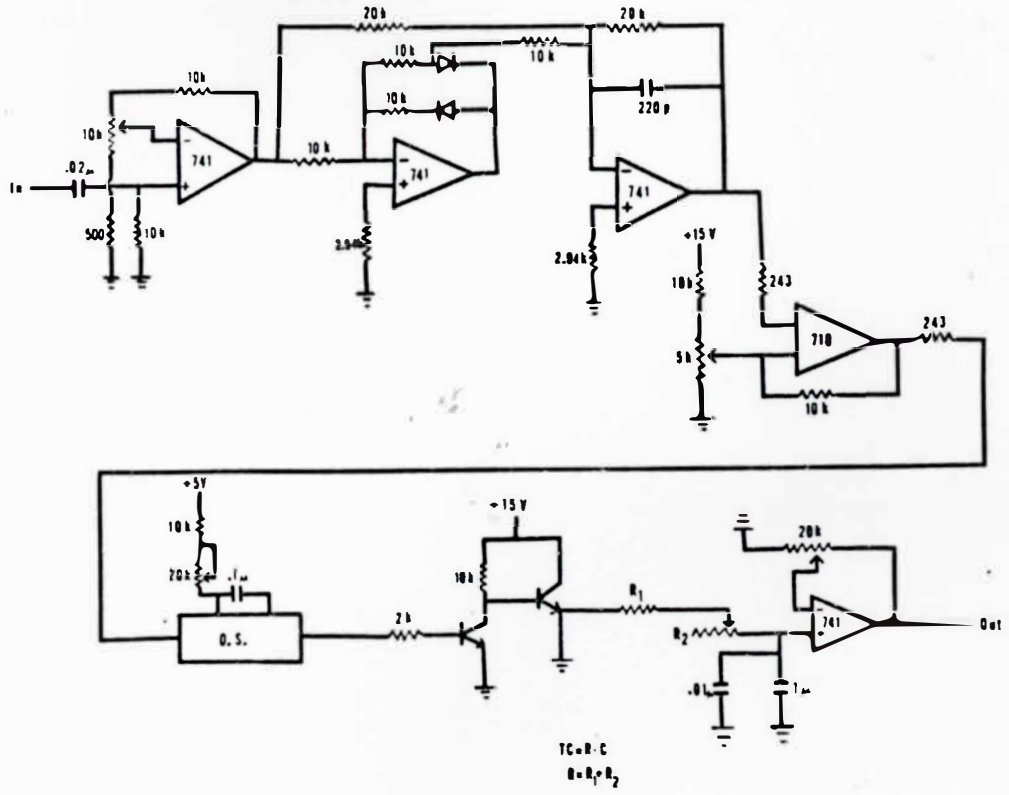
0.9% Saline in deionized water

Solution II

10% Acetic acid

0.9% Saline in deionized water

Appendix 5. Schematic Diagram of Integrator Circuit



Appendix 6

F-values for Linear and Curvilinear Correlations between Neural Response Amplitudes and Successive Trials

Inferior Colliculus

Animal #	Tone I		Tone II	
	F (linear)	F (curvilinear)	F (linear)	F (curvilinear)
32 I_L	7.38*	<1	6.23*	-.366
32 I_R	4.64*	-.17	26.26*	4.03*
24 I	4.03*	1.36	7.53*	2.33
23 I	2.24	1.51	--	--
20 I	1.60	1.35	--	--
21 I	<1	4.42*	8.84*	1.53

*significant at p <.05 level

Medial Geniculate

Animal #	Tone I		Tone II	
	F (linear)	F (curvilinear)	F (linear)	F (curvilinear)
31 I	6.23*	5.76*	5.08*	<1
35 I (on)	6.52*	1.40	4.22*	<1
35 I (off)	10.02*	1.09	3.69	<1
30 I	19.81*	1.05	1.61	16.48*
10 I	1.55	<1	8.37*	<1
7 I	2.98	1.42	<1	<1

* significant at p <.05 level

Auditory Cortex

Animal #	Tone I		Tone II	
	F (linear)	F (curvilinear)	F (linear)	F (curvilinear)
13	<1	9.88*	3.07	<1
10_R(on)	<1	2.64	14.20*	2.03
10_R(off)	<1	3.25	<1	<1
10_L	<1	3.49	1.52	1.73
17_L	<1	<1	<1	1.04
17_R	<1	1.14	<1	<1

*significant at p <.05 level

Polysensory Cortex

Animal #	Tone I		Tone II	
	F (linear)	F (curvilinear)	F (linear)	F (curvilinear)
10	2.84	<1	<1	<1
13_L	1.15	<1	10.17*	<1
13_R	<1	3.17	3.30	<1
11	2.09	1.32	<1	<1
16	5.06*	4.63*		
17	2.34	2.51	1.80	1.35

*significant at $p < .05$ level

Lateral Midbrain Reticular Formation

Animal #	Tone I		Tone II	
	F (linear)	F (curvilinear)	F (linear)	F (curvilinear)
30 I	5.97*	<1	<1	1.16
21 I	5.90*	13.78*	2.93	3.99*
31 I	1.59	2.94	7.41*	2.24
20 I	.91	5.95*	13.80*	2.20
23 I	1.89	<1	<1	1.95
35 I	<1	<1	<1	1.40

* significant at $p < .05$ level

Appendix 7

F-values for Linear and Curvilinear Correlations between MUA Activity Levels and Successive Trials

Medial MRF

Animal #	Tone I (lower)		Tone I (high)		Tone II (lower)		Tone II (high)	
	F_L	F_C	F_L	F_C	F_L	F_C	F_L	F_C
34	55.57*	-1.03	70.93*	-1.15	1.93	2.09	<1	<1
26	1	1.90	10.45*	1.54	1	<1	4.20*	<1
3	9.94*	1.16	21.57*	2.97	1	1.54	<1	<1
7	8.91*	<1	1.49	2.53	1	<1	<1	1.60
1	27.98*	-4.58*	<1	8.38*	15.57*	17.98*	6.40*	6.83*
37	1.32	1.04	19.36*	1.54			29.47*	2.63

F_L - linear

F_C - curvilinear

* significant at $p < .05$ level

Medial Septal Nucleus MUA

Animal #	Tone I (lower)		Tone I (high)		Tone II (lower)		Tone II (high)	
	F_L	F_C	F_L	F_C	F_L	F_C	F_L	F_C
1	<1	-2.80	58.65*	<1	3.30	2.83	7.79*	<1
37	47.29*	-5.00*	26.20*	1.65	13.89*	1.93	6.73*	1.85
3	26.0*	5.12*	39.62*	2.74	16.49*	<1	15.98*	<1
5	1.99	3.29	1.23	13.67*	<1	<1	<1	1.19
35	33.73*	4.27*	34.60*	<1	1.96	<1	<1	<1
4	9.16*	<1	<1	1.45	9.36*	1.61	4.76*	<1

F_L - linear

F_C - curvilinear

* significant at $p < .05$ level

Dorsal Hippocampus

Animal #	Tone I (lower)		Tone I (high)		Tone II (lower)		Tone II (high)	
	F_L	F_C	F_L	F_C	F_L	F_C	F_L	F_C
32	5.26*	-1.19	5.41*	2.57	18.05*	2.11	<1	21.50*
34	65.11*	<1	6.53*	<1	3.76	<1	<1	<1
4	14.97*	<1			<1	1.51		
9	2.79	2.80	<1	2.17	1.24	<1	2.32	2.85
8	<1	<1	<1	<1	<1	<1	1.59	7.51*
5	2.74	8.34*	4.33	1.76	2.60	<1	3.15	<1

F_L - linear

F_C - curvilinear

* significant at $p < .05$ level