ELECTROPHYSIOLOGICAL AND BEHAVIOURAL STUDIES OF HABITUATION

by

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PUBLICATIONS

The following publications have arisen out of the work reported in this dissertation:

(1) DUNLOP, C.W., WEBSTER, W.R. and SIMONS, L.A.
Effect of attention on evoked response in the classical auditory pathway, Nature, 1965, 208, 1048-1050.

(2) WEBSTER, W.R. and DUNLOP, C.W.
Unanesthetized Cats, Auditory Evoked Potentials and Test Cage Acoustics: A Methodological Study. J.Aud.Res., 1965, 5, 11-25.

(3) WEBSTER, W.R., DUNLOP, C.W., SIMONS, L.A. and AITKIN, L.M.
Auditory Habituation: A test of a centrifugal and a peripheral theory, Science, 1965, 148, 654-656.

(4) DUNLOP, C.W., WEBSTER, W.R. and RODGER, R.S.
Amplitude changes of evoked potentials in the auditory pathway of unanesthetized cats during acoustic habituation, J.Aud.Res., 1966, 6, 47-66.

(5) WEBSTER, W.R.
Auditory Habituation and Barbiturate induced neural activity, Science, 1969 (in press)

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ABSTRACT

Responses to repetitive stimulation are found to decrease in frequency and amplitude over time. Similar habituation effects have been reported in evoked potentials recorded in the auditory pathway of cats, although some later studies have not confirmed these initial findings.

The first set of experiments examined the relationship of auditory evoked potential habituation (AEPH) and stimulus variables known to determine behavioural response habituation (BRH). Decrements in evoked potentials to repetitive stimulation did not follow typical habituation functions.

The second set of experiments tested various theories which have been put forward to explain the decrements. Explanations based on conditioning of the middle ear muscles were rejected and explanations based on either reticular formation inhibition or cortical inhibition were considerably weakened. The only theory supported was one explaining the decrements by inhibition intrinsic to the recording area. A third set of experiments supported predictions from this theory and some evidence indicated that the intrinsic inhibition could be postsynaptic inhibition at the level of the thalamus.

A fourth set of experiments examined the effect on auditory evoked potentials of an animal attending to a stimulus in another modality. It was found that previous reports of a decrement during attention were based on an artefact of movement. However, attentive effects were observed at the thalamus during the presentation of aversive stimuli.

It was concluded that the sensory correlate of habituation might be based on a small number of specialised cells, whose function would not be detected by the evoked potential technique. It was also concluded that the evoked potential study of attentional mechanisms should concentrate on determining whether such effects were pre- or postsynaptic in nature.

CHAPTER 1

1.0 Introduction and Aim

One of the most important and enduring problems for psychology concerns selective perception. The solution of this problem will occur when psychologists can state the necessary and sufficient conditions under which an organism will respond to one stimulus and not to other stimuli in the perceptual field. One way of describing this sort of behaviour is to say that the organism attends to a certain stimulus and does not attend to the others in the situation.

The use of the word "attention" carries with it so many connotations from everyday usage that it is easy to feel that an explanation has been achieved when an instance of selective perception is attributed to attention. The real and vital problem of finding the conditions under which the behaviour occurs are not advanced by such an explanation. In fact, the use of the word attention was for many years avoided by psychologists (Hall, 1966). It is only recently that attention has been dealt with in psychology in terms of more formal operational definitions (Buckner and McGrath, 1963; Deutsch and Deutsch, 1963; Horn, 1965; Trabasso and Bower, 1968).

Concurrent with the revived interest in attention by psychologists, there has been an increasing interest shown, both by psychologists and by physiologists, in the elucidation of the physiological mechanisms responsible for this type of behaviour. In general, these studies have involved sensory evoked potential responses and the behaviour of these responses in two experimental situations.

In the first of these situations, an animal attends to a stimulus, attention being defined as occurring when the organism directs his receptors towards a stimulus. Sensory evoked responses are recorded during this behaviour, either from the pathway excited by the stimulus (Horn, 1960), or from another sensory pathway (Hernández-Peón, Scherrer and Jouvet, 1956). In both cases, a reduction in evoked response amplitude is observed. The second case has been interpreted as being due to a peripheral gating mechanism which blocks or reduces sensory input. The first case has been interpreted

as being related to searching behaviour rather than being due to a peripheral gating mechanism.

The second situation is one in which an animal ceases to respond to a repetitively presented stimulus which originally evoked a response. The animal is described as no longer attending to the stimulus. Sensory evoked potential responses are recorded during the presentation of repetitive stimuli and evoked potential amplitude is found to fall progressively (Hernández-Peón, Jouvet and Scherrer, 1957). This reduction is attributed to the operation of a peripheral gating mechanism, and this mechanism is put forward as an explanation of the animal ceasing to attend to the stimulus. More recently, both the gating mechanism interpretation and the validity of the basic evidence have been challenged for each situation (Horn, 1965; Thompson and Spencer, 1966).

The general aim of the experiments reported in this dissertation was to examine the relationship of the amplitude of auditory evoked potential responses both to stimuli in the non-attending (habituation) situation and to stimuli in the attending (directing of receptors) situation.

1.1 Habituation

The first part of this dissertation is concerned with the problem of habituation of evoked potential responses recorded in the classical auditory pathway of unanaesthetised cats. There is a considerable body of literature dealing with the concept of habituation (Harris, 1943; Thompson and Spencer, 1966; Glaser, 1966). This term refers to the progressive decrease in the response to any regularly repeated stimulus. Habituation occurs in widely different systems: protozoa, bacterial cultures, and systems based on a nervous system (Harris, 1943). This suggests that the determinants of habituation will vary markedly from system to system. Ashby (1959) has proved by formal analysis that all systems made of parts that are rich in states of equilibrium will show habituation. This entropy-like process suggests why habituation occurs over such a wide range of systems. Over and above this general process, it seems as if there have developed highly specialised mechanisms which will rapidly and efficiently show habituation or response decrement (Ashby, 1959).

The experiments reported in this part of the dissertation are concerned with the problem of whether such a specialised mechanism is present in, or acts upon, the auditory pathway. In the next section the characteristics of habituation displayed by intact vertebrates in response to repetitive stimulation will be examined. This will be followed by an analysis of the evidence for such a system acting on the auditory pathway.

Before the characteristics of habituation in intact vertebrates are discussed, an experiment by Prosser and Hunter (1936) will be examined in detail to clarify some of the general issues connected with the use of the term habituation. Prosser and Hunter studied the extinction of the startle reflex and the extinction of spinal reflexes in the white rat. It is the work on the startle reflex that is of major importance in the present context. Prosser and Hunter restrained each animal in a bag which left the animal's legs free. Each animal was also loosely tied to a table. It was observed that a brief click would produce an unconditioned startle response in their animals and that the continual presentation of this stimulus every 15 seconds led to a reduction in the occurrence of the response. Eventually the animals ceased to respond. This period of zero responding often lasted for minutes, and was described as the extinction of the unconditioned reflex.

In addition to recording the molar response, Prosser and Hunter recorded unit muscle potentials from the legs of their animals. These responses were correlated with the molar response in that they became much reduced in number until ultimately no units responded. As these units dropped out there was no change in latency. It was argued that "the effect is as if the threshold for different units became higher, each dropping out at some level without undergoing any change in its time relations" (Prosser and Hunter, 1936, p.610).

Weak auditory stimuli were paired with a shock to the leg. The auditory stimuli did not elicit the startle response, but in many cases did so after a number of pairings. This was described as conditioning of the response, although later in the paper it was suggested that this might not be true conditioning but more akin to facilitation or sensitisation (Kimble, 1961). Their explanation was that the shock somehow raised general excitability of "centers involved so that a sub-threshold stimulus becomes

and then remains effective" (p.613). These "conditioned reflexes" showed a similar extinction pattern to the unconditioned response. Both types of response recovered with time; moreover, extraneous stimuli removed the extinction so that the response reappeared. Faster rates of stimulation (one per two second or one per five second) were found to hasten extinction and there was faster extinction when weaker stimuli were employed.

Three general issues related to the term habituation are raised by the work of Prosser and Hunter. The first involves a clarification of terminology and the other two are related to the nature of the habituation process:

1. Prosser and Hunter have used the term extinction to describe the decrement observed in both the original startle response and the "learned" startle response. An important distinction can be made if the term extinction is considered to apply only to the decrement in learned responses when reinforcement is withheld, the term habituation being used, in contrast, to describe decrements in a wider class of responses, such as unconditioned responses or unlearned responses and responses whose learning history is unknown. This distinction is not meant to imply that extinction and habituation are unrelated. Indeed, Prosser and Hunter's study suggest that they might be based on the same mechanism.

2. It could be argued that the decrements observed by Hunter and Prosser are based on either sensory or neuronal adaptation. However, Hunter and Prosser pointed out that the work of Davis et al (1934) has shown that rates of stimulation as slow as one every 10 or 15 seconds do not produce adaptation in the auditory pathway. By this temporal criterion, it does seem to be possible to distinguish habituation from adaptation.

3. It is possible that the decrements are due to some sort of fatigue process, in the sense of accumulation of waste products or the depletion of reserves. Prosser and Hunter (1936, p.616) adduced two pieces of evidence against this position: faster decrements occur with weaker stimuli, and extraneous stimuli can abolish the decrement. These two observations are incompatible with an explanation in terms of fatigue.

On the basis of these three points, habituation will be defined in this dissertation as the reduction in size of responses in this wider class,

during a period of repetitive stimulation. This reduction will be taken to be based on a central process, so that instances of adaptation, fatigue or any peripheral failure in either receptors or effectors will not be regarded as instances of habituation.

1.1.1 Behavioural Response Habituation

Harris (1943) reviewed the existing literature on habituation and showed that this response decrement occurs throughout the phylogenetic scale. The nature of the response decrement becomes more complex as organisms become more complex. For example, response decrements occur that last for hours and are produced by a small number of stimuli separated by intervals as long as two hours (Harris, 1943).

In the highest forms, intact vertebrates, the habituation decrement has complex determinants. There have been a number of recent attempts to specify the parameters of the relations between stimulus and response in these decrements (Webster, 1964; Thompson and Spencer, 1966). The latter authors have called these response decrements "behavioural response habituation" (BRH). This apparently redundant name is helpful in distinguishing the process from various forms of neurophysiological response habituation.

The following sections set out the main parameters of BRH under three categories. They are parameters related to the development of, the recovery from, and the disruption of, BRH.

1.1.1.1 Development of BRH

(a) Stimulus Interval

Almost by definition, a situation in which habituation occurs is a situation involving more than one stimulus presentation. It may involve only a few stimuli or it may involve a large number. This suggests that an important parameter for the development of BRH is the time between successive stimuli. In most cases (Harris, 1943), it appears that faster rates of stimulation produce faster development of BRH. This rules out the possibility that habituation is solely a function of the number of stimuli presented. This factor could interact with rate of stimulation, but a large number of

stimuli presented at a slow rate would not produce the same degree of habituation as the same number presented rapidly.

The importance of stimulus interval raises a problem. We have already seen that a temporal criterion is employed to distinguish habituation from sensory and neuronal adaptation. But if faster rates of stimulation produce a quicker growth of BRH, then it can be asked: exactly where does habituation stop and other processes commence? There has been a tendency for theorists in this field to ignore this point (Harris, 1943; Thompson and Spencer, 1966). It is not an easy problem to solve since some response systems habituate rapidly to stimulus intervals which are far too long for processes such as adaptation (Harris, 1943). It suggests that a general statement about the parameter of stimulus interval might need to be qualified to take into account different types of response systems.

(b) Stimulus Intensity

There is usually more rapid habituation with weaker stimuli (Harris, 1943; Thompson and Spencer, 1966). However, there have been a few reports of habituation decrements that appear to be independent of stimulus intensity (Harris, 1943). There are no reports of faster habituation with stronger stimuli and in most cases there is virtually no habituation to intense stimuli. This is an important observation, because it is well established that pronounced receptor and neuronal adaptation occur to intense stimuli (Osgood, 1953). Thus an important criterion for distinguishing behavioural response habituation could be based on a joint function of stimulus intensity and rate of stimulation. A response system which shows more rapid decrements to shorter stimulus intervals could only be confirmed as habituation if it also gave more rapid or deeper habituation to weak stimuli given at short intervals compared with strong stimuli at short intervals. This particular implication does not appear to have been tested in studies of BRH.

(c) Stimulus Duration

The influence of stimulus duration has not been studied systematically (Harris, 1943; Thompson and Spencer, 1966). Most experiments have involved stimuli of brief duration (Such as taps on a plank of wood, clicks, briefly

presented shadows). One can assert that by definition the stimulus duration must at least be shorter than the interval between stimulus onsets. However most experimenters have kept the duration very brief to avoid the possibility of introducing sensory or neuronal adaptation into the decrement.

(d) Repeated Sessions

The rapidity with which BRH develops increases as a function of the number of habituation sessions (Thompson and Spencer, 1966).

(e) Level of Development

The most common finding reported is that a response habituates to a zero level (Harris, 1943; Thompson and Spencer, 1966). For example, the startle response of a rat to a click eventually ceases altogether (Prosser and Hunter, 1936). In some cases, the response may never entirely disappear but become asymptotic. This means that the experimenter counts the number of times on which the response fails to appear. This is not a very sensitive measure of habituation. It is possible that more sensitive measures (such as response amplitude or response latency) might have revealed response changes before the first non-occurrence was observed. It is probable that the lack of studies using these measures is due to the difficulty in obtaining such records from intact organisms, and the relative ease with which frequency measures can be employed.

Thompson and Spencer (1966), using an acute mammalian spinal preparation, have been able to obtain response amplitude habituation measures of the strength of the flexion reflex of the tibialis anterior muscle. It may be concluded that the final level of habituation will depend largely on the type of measure employed and no completely general finding is available.

(f) The Form of the Development Function

It is generally observed that when the response decrement is plotted as a function of time, there is a rapid initial fall followed by a progressively slower fall. Harris (1943, p.416) claimed that this negative exponential function is a distinguishing feature of BRH. It is, of course, a function that applies to measures of some cases of receptor and neuronal adaptation, and thus it is hard to see it as a distinguishing characteristic of BRH.

(g) Stimulus Generalisation

There is some evidence that habituation to one stimulus generalises to other similar stimuli which have not previously been presented (Hunter and Prosser, 1936).

1.1.1.2 Recovery of BRH

(a) Spontaneous Recovery

If repeated presentation is discontinued for some time, then the response will recover over time. The rate of the recovery depends on many variables and it does not seem possible to categorise types of habituation on the basis of recovery time (Thompson and Spencer, 1966). The use of the name spontaneous recovery is not very helpful. Other processes such as adaptation also recover when the stimulus is not presented. This is a characteristic which would only distinguish whether the determinants of habituation were based on a form of learning. That is, a response decrement based on learning would not disappear with the mere passage of time (Kimble, 1961).

(b) Over-Habituation

When the response has been habituated to a zero level and presentation of the stimulus is continued, then it appears that recovery is retarded compared with responses that are not over-habituated (Harris, 1943; Hunter and Prosser, 1936).

1.1.1.3 Disruption of the Decrement

(a) Dishabituation

The presentation of another stimulus can lead to the immediate restoration of the habituated response. This disruption is called dishabituation (Thompson and Spencer, 1966). It appears to be present more often in the response systems of higher organisms (Harris, 1943). This would appear to be an important way to distinguish habituation from sensory and neuronal adaptation, because extraneous stimuli do not abolish adaptation.

For example, if subjects adapt to a smell or adapt to an intense sound, then there is no evidence for disadaptation by extraneous stimuli.

(b) Repeated Dishabituation

The dishabituation effect can be itself habituated (Thompson and Spencer, 1966). That is, repeated presentation of the dishabituating stimulus leads to less and less dishabituation. This decrement in dishabituation appears to follow a negative exponential function.

1.1.1.4 The Relationship Between BRH and Neural Response Habituation

There has been a widespread renewal of interest in the phenomenon of habituation as a result of the development of techniques for recording from sensory pathways in awake, unrestrained animals. Thompson and Spencer (1966) have used the general term "neural response habituation" to refer to these studies. They do not distinguish between studies using macro electrodes recording evoked potentials and those using microelectrodes and recording unit potentials. It would seem better to refer to the first group of studies as sensory evoked potential habituation and the latter as sensory unit potential habituation. When a reference is made to a particular sensory system, it will be described as, for example, auditory evoked potential habituation (AEPH). This could be regarded as a pedantic distinction, but with the introduction of new techniques such as multi-unit recording (Buchwald et al, 1966) it becomes necessary to make quite clear the class of response that is being studied.

It also becomes important to specify the characteristics of BRH because the more recent studies have been concerned to show that sensory evoked potential habituation follows similar stimulus-response relationships (Hernández-Peón, 1960 ; Desmedt, 1960). This renewed interest is also based on a new theoretical context. The work has been stimulated, not by an interest in the neural foundations of habituation, but rather by an interest in the centrifugal control of sensory pathways. The habituation paridigm has been used to show that there is centrifugal control exerted as peripherally as the first sensory synapse (Hernández-Peón, 1960). It is critical to stress this theoretical context because most studies have taken the following form.

Electrodes are implanted in a sensory pathway and the effects of repetitive stimulation on evoked responses are observed. If a decrement, usually in amplitude, is obtained, then attempts are made to see whether this decrement has the characteristics of BRH. If the characteristics are isomorphic with those of BRH, then it is argued that BRH is based on a centrifugal mechanism (Hernández-Peón, 1960). This conclusion is only justified if there are concurrent recordings of a molar response and the neural response. However, there have been no studies of this type reported in the literature.

Even if such a correlation study were carried out, the conclusion would only hold if an important condition is assumed. That is, that the isomorphic relationships hold throughout the neural chain between stimulus and response. This point can be made clear by again considering the study by Prosser and Hunter (1936). It was mentioned earlier that Prosser and Hunter recorded unit potentials from various leg muscles (usually the gastrocnemius) concurrently with the startle response. On the basis of the very short latencies of some of the units (15 msecs), they rejected the notion that the cerebral cortex was involved in the extinction of the startle response. According to Prosser and Hunter, the most likely pathway for the startle response was: cochlea, eighth nerve, cochlear nucleus, inferior colliculus, reticular nucleus and the mid brain, reticulo-spinal tract, anterior horn cells and motor nerves to the leg muscles. If one allows for a synaptic involvement of the superior olive complex, this pathway could contain at least five synaptic interruptions. A conclusive demonstration of the centrifugal basis of BRH would need evidence of the behaviour of each stage in the pathway.

However, the search for neural changes that are isomorphic with all the characteristics of BRH could obscure important information. For example, if an evoked potential recording were taken in the inferior colliculus of the above pathway, and if the response decrement had only <u>one</u> characteristic similar to those of BRH, then what conclusion could follow? One possible conclusion is that the characteristics of BRH (a <u>molar</u> response) might be based on the individual contribution of a number of parts of the pathway. That is, partially negative evidence need not imply a lack of any relationship with BRH. The next section examines the evidence that AEPH has characteristics that are isomorphic with characteristics of BRH.

1.2 Habituation of Auditory Evoked Potentials

A group of studies carried out by Hernández-Peón and his colleagues initiated this renewed interest in habituation (Hernández-Peón and Scherrer, 1955; Hernández-Peón, Jouvet and Scherrer, 1957). They observed that "monotonous repetition of an acoustic stimulus resulted in persistent diminution and eventual disappearance of the evoked potentials recorded from the dorsal cochlear nucleus" (Hernández-Peón, 1960, p.102). Hernández-Peón pointed out the similarity of the response decrement to BRH and went on to infer that the sensory decrement could be part of the basis of BRH. Although he did not set it out in this form, his evidence can be grouped into the following three categories:

(1) The development of sensory evoked habituation occurred to well spaced stimuli and eventually achieved a zero response level with much waxing and waning of the decrement.

(2) The decrement recovered after a period of rest.

(3) The decrement could be disrupted by
   (a) the presentation of extraneous stimuli,
   (b) the repeated association of the auditory stimulus with a painful stimulus (for example an electric shock),
   (c) the administration of barbiturate anaesthesia.

Most of these features of sensory evoked habituation appear to agree with characteristics of BRH. The most striking aspect of the observations is that the sensory evoked potential recorded in the first synaptic area of the auditory pathway eventually disappeared.

The results were soon confirmed by Galambos et al (1955), who found marked decrements in evoked responses at the cochlear nucleus of cats to repetitive stimulation. However, other studies (Huttenlocher, 1960; Worden and Marsh, 1963; Marsh and Worden, 1964) have failed to confirm the original findings concerning sensory evoked habituation at the cochlear nucleus.

Such divergent results suggest that it would be profitable to examine the experimental procedures and stimulus conditions in some detail to determine whether their apparent discrepancies can be accounted for by procedural differences, or to suggest possible experiments to resolve the

issue. This conflict of evidence has led Thompson and Spencer to conclude after a careful analysis that "there is as yet no consistent agreement regarding the occurrence of habituation in the sensory systems of the working brain" (1966, p.22).

1.3 Experiments on Habituation in the Auditory Pathway

1.3.1 Cochlear Nucleus

The first experiment (Hernández-Peón et al, 1956; Hernández-Peón and Scherrer, 1955) has already been mentioned, but there are a number of other features of this experiment to which attention should be drawn. It is stated that eleven cats were implanted, two with bilateral cochlear nucleus (CN) placements and nine with unilateral placements, giving a possible maximum of 13 placements. However, from the text it is not possible to determine either how many placements were successful or how many contributed towards the results. The specification of stimulus parameters is not complete. It is stated that stimuli were delivered at intervals of two seconds and more, but in most cases it is impossible to tell exactly the rate used in a given experiment. The auditory stimuli were produced from trains of square wave pulses with a duration which varied from ten to 100 msecs. Once again, which duration applies to a particular set of results is not indicated. By closely studying the records, it is possible to estimate that the experimenters used clicks of 10 - 20 msecs and "tone" burst of 100 msecs. The specification of the frequency of these bursts is also vague, being "in arbitrary steps (from 1,000 to 5,000/sec)" (p.145), without reference to the exact steps employed. The intensity of the stimuli was not measured at all, although it was kept constant. It should also be pointed out that it is not possible to obtain pure tone stimuli from sounds generated by trains of square wave pulses, because transients are induced by the inability of the speaker to follow the onset of square waves. An instrument such as an electronic switch is necessary.

The reporting of the results is also inadequate. There are a few oscilloscope traces but no attempt at quantification. Habituation is said to become more rapid with repeated sessions but no evidence is presented.

More trials are said to be required to habituate to more intense sounds, but no evidence of the range of intensities is provided. Little weight can be given to the results on stimulus generalisation owing to the confounding of pure tones with transient sounds.

It can only be concluded that sensory evoked habituation occurred in the CN of an unknown number of cats to stimuli of varying rates and durations and of unknown intensities. There appears to be dishabituation by extraneous stimuli, barbiturates and lesions, but the number of placements involved is unknown. Although there is mention of variability in the data, there is no attempt to apply statistical methods.

The second experiment was carried out by Galambos et al (1955). Electrodes were implanted in the CN and other auditory and non-auditory areas of ten cats. The results showed habituation of the CN evoked potentials to click stimuli. When the stimulus was paired with shocks administered through electrodes on the chest, there was "dishabituation" and the response became larger. Once again there is inadequate reporting of results and relevant parameters. No evidence is presented as to how many placements showed the effect and what sort of variability was present. The clicks, of unstated intensity and duration, were presented at a rate of 1/3 seconds for periods of many days and weeks. A control study on an unstated number of animals under muscular paralysis due to Flaxedil showed that the effects were not due to muscular components. That is, both habituation and dishabituation were observed under these conditions. The presentation of the results was also incomplete. It consisted of oscilloscope traces, which did not show both pre- and post-habituation levels. The size of the habituated response was only a little greater than the noise level. There was no attempt to apply statistical methods to the data. It is realised that this paper is a first report of the work, but it has not been followed up by a more detailed account.

In a study that was mainly designed to investigate the effects of arousal on click responses in the reticular formation, Huttenlocher (1960) failed to find habituation in the CN of seven cats to clicks given at the rate of 1/3.2 seconds over a period of an hour. The duration of the click is not mentioned and an indirect measure of stimulus intensity is provided

(for example 40 - 50 db above CN threshold). No habituation data are provided, but this is understandable since this was a minor aspect of the work.

Marsh et al (1961) reported marked habituation of evoked potentials recorded at the CN of two cats to click stimuli given at the rate of 1/10 seconds. They also recorded from other auditory areas and their results will be discussed in later sections. The intensity of the stimulus is given as 80 - 90 db, but it is not indicated whether this is a sound pressure level or how the measurements were made. The stimulus duration was not reported. The stimulating session lasted five to seven days. At the end of the period 50 responses were recorded and compared with a control consisting of the first 50 responses. The differences obtained were significant (by a t test) at the .001 level, but there appear to be separate t tests on the data from each individual cat, a procedure which would be difficult to justify statistically. The response of one placement decreased to noise level, a result similar to Hernández-Peón's finding. However, close examination of the published records of the control responses reveals that they were barely visible. That is, the size of the response above noise level would not justify its use as an indication of the pre-habituation level. This is even more apparent when the authors reported marked dishabituation by pairing the click with about ten air puffs to the face. The size of this doubtful response becomes two or three times the original level. Such a marked change might indicate a loose electrode. It is concluded that this confirmation of Hernández-Peón's findings is not entirely convincing.

The next study represents a marked improvement in experimental design and stimulus control. Worden and Marsh (1963) examined the effects of presenting repetitive clicks for six hours at rates of 1/1 sec and 1/10 sec. Ten placements were observed at 1/1 sec and 12 placements at 1/10 sec. A 0.5 msec click of 70 db intensity was used. It is appropriate to point out here that their method of measuring click intensity is invalid. They reported "using the General Radio sound level meter ("fast B" scale) with the microphone in a central reference position" (Worden and Marsh, 1963, p.867). This procedure is invalid because the inertia of a sound level meter will not allow accurate sound pressure measurements of clicks. Accurate peak measures can be made by using an impact noise analyser in conjunction with a sound level meter or by calibrating an oscilloscope to

measure sound levels.

Worden and Marsh failed to find significant habituation at the CN to either rate of stimulation, basing their conclusion mainly on the variability of the results. For example, in one animal stimulated at 1/1 sec, one placement showed a decrement over six hours of 15 - 20% while the other placement showed an increase of 7 - 10%. This is an interesting observation, but it does not rule out a conclusion that habituation has occurred. Two graphs are shown which give the individual plots for each placement at each rate. If one examines these closely there might be some evidence for a rate effect. For example, six out of ten placements show decrements at 1/sec and the remaining four show increments. In contrast, only four out of 12 placements show decrements at 1/10 sec and eight out of 12 show increments. The statistical treatment did not allow a statement that there was a difference between the two conditions, yet this seems to be the hypothesis under test. It is clear, however, that Worden and Marsh failed to find the marked decrements (to noise level) previously reported (Marsh et al, 1961; Hernández-Peón et al, 1955, 1956). One further methodological observation should be made. The first 50 responses were used as the control, which raises the possibility that if the decrement is rapid, then this control could hide a small but significant decrement.

Worden and Marsh (1963) were able to obtain bipolar and monopolar records from the same electrodes. They found that two adjacent monopolar recordings could lead to amplitude changes in the opposite direction. They were unable to find any correlation between recording site in the CN and the direction of amplitude change. Worden (1967) has stressed that these observations are difficult to reconcile with any theory that habituation is based on loss of attentiveness due to loss of stimulus novelty.

Marsh and Worden (1964) carried out another study in which they failed to find habituation at the CN, to repetitive presentation of a click at 1/10 sec for six hours. Data are reported on ten placements using a one msec click of 65 db re 2×10^{-4} microbar. Once again, it is difficult to tell whether this is a valid figure for intensity because the methods used to obtain the measurement are not stated exactly. The results are inconsistent, in that five placements show decrements and five placements show increments.

The largest amplitude change is 10% of the control.

Marsh and Worden (1964) used small earphones mounted on the head of the animal to deliver the stimuli. This procedure reduced the amount of evoked response variation due to change in position in the acoustic field. It is important to note that in no other study has there been any attempt to control this type of variation. In fact, no data are provided in the other studies about the variation in stimulus intensity in the range of movement of the experimental animal. Without direct control of this variation, this information is critical before any interpretation of amplitude change can be made. This point will be further discussed in a later section.

It is difficult to reach many firm conclusions on these studies. It is clear that none of the later studies have been able to confirm habituation decrements to noise level. When a decrement is observed the maximum value is of the order of a 20% reduction compared with control amplitudes. Most studies have incorrectly measured stimulus intensity and it is probable that the true intensities were greater than those reported. This could be a confounding variable given that there is less habituation with more intense stimuli. In brief, it is not possible to point to any one condition or set of conditions that differ between the experiments showing conflicting results. It looks as if the true decrement to repeated stimulation is small, but this might still be an important contribution to any BRH.

1.3.2 Superior Olive and Inferior Colliculus

Marsh et al (1961) reported data on habituation, with eight superior olive complex placements, to click stimuli given at 1/10 sec. They reported that four placements showed decrements averaging 11%, two showed increments averaging 39% and two showed no change. They could not find any relationship between either direction or extent of this change with respect to the nuclear masses in this complex region of the auditory pathway. There were marked increments in amplitude at all placements when the click was paired with an air puff.

Desmedt (1960), in a review paper, reported marked habituation in the

superior olive to clicks given at the rate of 1/3 sec. The clicks were described as "30 db above threshold" (p.153) so it is impossible to determine the sound pressure level used. No information is provided about stimulus duration and the number of placements involved or the number showing the effect. Oscilloscope recordings are shown, and the selection presented demonstrates very marked habituation, almost to noise level. When extraneous stimuli were presented the response amplitudes showed an immediate return to near pre-habituated levels.

Marsh et al (1961) also reported data on seven inferior colliculus (IC) placements. Four placements showed increments averaging 37% and three showed no change or no significant increase. There were no amplitude decrements. The records provided in the paper have a very poor signal-to-noise ratio, and in the control sample it is difficult to distinguish the response. When the clicks were paired with an air puff there were marked increases in amplitude in four placements and no change in two placements. No information is provided on the fate of the seventh placement. When the air puff is removed and the conditioned effect is extinguished, there were again amplitude reductions, though "not always to preconditioning habituation levels" (p.229).

Dunlop, Webster and Day (1964) reported habituation at five IC placements to 20 msec clicks presented at the rate of 1/2 sec. Three intensity levels were employed (85, 95 and 105 db). The degree of habituation was an inverse function of stimulus intensity.

As with the CN, there is a confusing picture. Some placements show decrements, some increments and sometimes there is no change. Stimulus conditions vary greatly between experiments and there does not appear to be any easy explanation for the observed discrepancies. On the basis of these results it is difficult to relate AEPH at either nucleus to BRH.

1.3.3 Medial Geniculate

Al'tman (1960) reported marked decrements (20 - 42%) in the amplitude of evoked potentials to the presentation of clicks at the rate of 1/3 sec. The duration of the click was 1 msec and the intensity is given as "40 db above the threshold for perceptible response" (p.618). After administration

of barbiturates, prolonged stimulation did not produce habituation. In some cases "spontaneous" recovery occurred over a period of 2.5 - 3 hours, and in some cases there was little restoration after three hours. However, no data are provided to support these claims. Al'tman also reported dishabituation by a painful stimulus using an unusual technique. Shock was administered 30 - 60 sec after the auditory stimulation ceased. Then there was a further period of two to three minutes without any form of stimulation. When the auditory stimuli were reintroduced, the amplitude was found to have recovered to the pre-habituated level. This design confounds effects of "spontaneous" recovery with dis-habituation. This could have been avoided by continuing to present the auditory stimulation. Another unusual feature of this experiment was that Al'tman used one loudspeaker for the sustained presentation at 1/3 sec, but used another for the test stimuli to which electrophysiological responses were recorded. The "sustained" loudspeaker is described as a low frequency speaker situated 50 cm above the restrained animal's head, and the test loudspeaker is a higher frequency speaker situated 10 cm above the head. The reporting is not clear, but it would appear that different stimuli are being used and the experiment becomes in effect a study of stimulus generalisation. Thus it becomes rather difficult to relate this study to the rest of the literature.

Marsh et al (1961) stimulated five MG placements with clicks at 1/10 sec and found marked amplitude increases at three placements (53%) and no change at two placements. Once again the published records of the control responses show very poor signal to noise ratio. As with other placements, the pairing of the clicks with air-puffs led to even greater increases in amplitude over control amplitudes.

Guzman-Flores et al (1960) reported marked habituation in evoked response amplitude at the MG in an unstated number of placements. Their description of the stimulus is as follows: "each stimulus of ten milliseconds long, at a frequency of 1,000 per second, were given at five second intervals during three hours" (p.136). No information is provided about stimulus intensity. Using an encéphale isolé preparation, with bilateral severance of the tendon of the tensor tympani muscle, habituation was reported as not occurring, but the photographs of oscilloscope records still indicated a decrement in the MG response. Later, their results showed habituation at

the MG of an encéphale isolé preparation which is abolished by the a administration of Flaxedil.

In another experiment, Alcaraz, Pacheio and Guzman-Flores (1962) obtained marked habituation in the amplitude of MG evoked pesponses to clicks given at the rate of 1/5 sec. The clicks were of 50 msec duration, but once again no information was provided about stimulus intensity. They report dishabituation by "stimulation of any other type" (p. 3), but they give no details. They failed to find habituation after the severance of the tendons of the tensor tympani muscles. In this case, their records appear to support this claim.

Jane et al (1962) observed some habituation in both the MG and the lateral geniculate responses to the simultaneous presentation of clicks and a stroboscopic flash at 1/sec. The click was of 0.2 msec duration and "the peak sound intensity of the click was not accurately measured, but it was estimated to be of the order of 80 - 90 db" (p.344). The method of "estimation" is not reported. Electrodes were implanted in three cats, but no information is provided on how many placements showed the decrement.

This analysis of habituation at the MG reveals the same pattern of conflicting results seen at other nuclei. The findings range from large increments to large decrements. In many of the experiments there was inadequate stimulus control, and it is not possible to determine how many subjects were used. Despite the variability in the data, there was little or no attempt to apply statistical techniques. As a result, it is difficult to account for the discrepancies by any single factor that might be different across experiments. It is also difficult to relate this work to BRH.

1.3.4 Auditory Cortex

A'ltman (1960) found considerable habituation of auditory evoked potentials in 11 cortical placements, with clicks given at the rate of 1/3 sec. When the clicks were presented 1 to 1.5 hours after injection of barbiturates, no habituation was observed. Dishabituation was obtained by the application of an electrical shock.

Marsh et al (1961) reported changes in evoked cortical responses from five placements in response to clicks given at a rate of 1/10 sec.

Three placements showed average decreases of 34% and two placements showed average increases of 32%. Once again there was a marked increase in amplitude over control levels when the click was paired with an air puff.

Guzman-Flores et al (1960) found marked habituation of auditory evoked potentials in an unstated number of placements to 1/5 sec clicks. Habituation was no longer seen when the tendons of the tympani muscle were cut or when paralysis by Flaxedil was induced. Alcaraz et al (1962) confirmed these results in an unspecified number of placements.

Moushegian et al (1961) observed habituation of cortical evoked responses at four placements to clicks presented at 1/10 sec over ten days. These animals had both the stapedius and tensor tympani muscles cauterized bilaterally. Dishabituation by pairing the clicks with an air puff also occurred. These results are in direct conflict with the results of Guzman-Flores et al (1960) and Alcaraz et al (1962). However, like other workers in this field, they have not described the relevant stimulus parameters of intensity and duration.

Marsh and Worden (1964) studied habituation at ten placements in the auditory cortex of cats. Decrements occurred at all placements (average 22% after six hours), but in non-alert animals the decrement was not apparent.

The results for the auditory cortex are a little more consistent than the results of other placements. In most cases, sensory evoked habituation occurs to repetitive click stimulation. There remains some conflicting evidence concerning the role of the middle ear muscles. Some of the features of BRH appear in habituation of cortical evoked potentials, but most of the experiments do not test the relationships in a systematic fashion.

1.3.5 Cochlea

Al'tman (1960) also implanted electrodes on the round window of ten cats. His records showed marked habituation in the N_1 component of the neural response recorded at the round window and no change in the cochlear microphone response. He found spontaneous recovery of this habituation and disruption by administering an electric shock. There was no decrement in the N_1 response if stimulation commenced 1.0 - 1.5 hours after injection of barbiturates.

Baust et al (1964) reported no systematic reduction in the amplitude of N_1 potentials to click stimuli given at the rate of 1/sec over eight hours. They did not specify how many placements showed this effect or the duration of the click. Bruno et al (1966) implanted electrodes on the round window of guinea pigs and found marked habituation of the N_1 response and the cochlear microphonic response to clicks of 0.3 - 0.5 msec duration. They omitted to mention how many placements showed the effect, although they reported operating on 45 animals. The sound levels of the clicks were 70 - 90 db as "measured with a General Radio sound level meter" (1966, p.25). As pointed out earlier, this is an incorrect method of measuring the sound level of transient stimuli. Habituation was also observed to tone pips of 50 - 150 msec duration. When an extraneous stimulus was introduced dishabituation occurred. The pairing of the click with an electric shock also increased the amplitude of the N_1 response and the cochlear microphonic.

One of the unusual features reported in this paper is that "the low signal-to-noise ratio prevents the detection of the potentials throughout the experiment, with single records or superposition (p.31). This is an unusual situation, since cochlear responses are usually quite large compared with gross recording levels higher up the auditory pathway. This might be due to their technique of partly removing the middle ear bones and introducing the sound source after the tympanic membrane through the superior recessus. To analyse the response they have used an averaging technique based on a photo-optic-electronic method (pp.25-26). This inability to record individual potentials of reasonable magnitude in comparison with the other studies suggests that the results of this study should be accepted with reservation. If normal transmission has been disrupted, as suggested above, then one should be cautious in extrapolating these results.

In summary, two main points can be made about the cochlear response and habituation. Firstly, there are fewer studies than in most other areas. This probably reflects the technical difficulties of implanting and maintaining chronic round window electrodes. Secondly, the existing evidence is similar to other areas, in that there are conflicting results and the control and the specification of stimulus conditions is not adequate. There is little convincing evidence for AEPH at the cochlea.

The above literature survey supports the conclusion of Thompson and Spencer that there is little "consistent agreement regarding the occurrence of habituation" (1966, p.22). However, such a variety of methods and stimulus conditions have been employed in studying the auditory pathway that it is difficult to judge whether the inconsistencies are in the phenomenon being studied or whether they are due to differences of method and conditions or indeed both. The problem is compounded by the fact that the literature in the field abounds with papers that simply do not report all the relevant variables. Even when the relevant variables are reported, the relationship between AEPH and BRH is not tested by systematic experiments. Thus is would seem that Thompson and Spencer's conclusion, as applied to the auditory pathway, needs to be tested by careful parametric studies. Another feature of this literature is its apparent isolation from classical studies of evoked potentials. This can be seen most clearly with regard to the effect of stimulus interval. There is evidence (Chang, 1959; Bishop, 1964) that a single stimulus can lead to evoked potential depression in the visual cortex and the lateral geniculate which will last several seconds. Yet some of the CN studies reported earlier (Hernández-Peón et al, 1965; Worden and Marsh, 1963) have used a repetitive stimulus interval of one second and two seconds. This suggests a possible confounding of the effects of habituation with refractory effects. It is true that these studies involved the CN, and there could be important differences related to either the visual system or the thalamus. However Al'tman (1960) has shown AEPH at the thalamus and the auditory cortex using intervals of three seconds.

The existing evidence for a relative refractory period in the auditory pathway is surprisingly limited. Tunturi (1946) reported a relative refractory period at the auditory cortex of 250 msecs. This figure is, of course, too short to explain AEPH, but repetitive stimulation could increase its value. In contrast, Rosenzweig and Rosenblith (1953), using cats anaesthetised with barbiturates, found that the recovery cycle of the auditory cortex in response to the second of two paired clicks to be much longer. They remarked "that the considerable duration of the cortical recovery function makes it inadvisable to crowd successive trials together. We spaced trials at lease one second apart, and, when the duration of recovery function warranted it, we used inter-trial intervals of two seconds and more"

(Rosenzweig and Rosenblith, 1953, p.15). A possible description of habituation could be the crowding together of successive trials. However, the objection being raised here is not that AEPH is necessarily a refractory type process, but that the existing studies have not even considered this possibility, let alone controlled for it.

Another example of the isolation of this work from the existing literature involves the use of barbiturates to remove AEPH (Hernández-Peón et al, 1955; Al'tman, 1960). There is a considerable body of evidence showing that barbiturates depress the recovery cycle of sensory pathways (Marshall, 1949; Chang, 1959). Rosenzweig and Rosenblith (1953) reported that increasing levels of barbiturate increased the recovery time of the auditory cortex. On the surface, this evidence conflicts with the observation that AEPH is abolished by barbiturates, because the abolition can be interpreted as response enhancement. It is, of course, quite possible that two independent mechanisms are operating, but it would need careful analysis to disentangle the effects. This possibility is never considered in the above papers.

Both of these points suggest that there may be a tendency to search for data from studies of the auditory pathway which fit the characteristics of BRH, a tendency that obscures possible simpler explanations of the results obtained. Other issues that are neglected in this literature involve the questions of what an evoked potential represents and what a change in an evoked potential represents. These problems will be discussed in the next section.

1.4 The Evoked Potential Technique and Auditory Evoked Potential Habituation

Evoked potentials have been defined by Chang (1959) as "the detectable electrical change of any part of the brain in response to deliberate stimulation of a peripheral sense organ, a sensory nerve, a point on the sensory pathway or any related structure of the sensory system" (p.299). This definition fails to distinguish evoked potentials from all-or-none unit action potentials and it refers to gross electrical recordings from a number of neurones by a macro electrode. Chang (1959, p.300) points out that evoked potentials usually have presynaptic and postsynaptic components. The

presynaptic component represents the arrival of impulses in axons and their terminals. (It would be possible to record an evoked potential that was purely axonal, but most evoked potentials recorded from the brain would have these two components.) The postsynaptic component represents the activities of the cells and their dendrites in the region of the electrode. When electrodes are implanted in areas of the auditory pathway such as the CN, the IC and the MG, the recorded potentials will have both presynaptic and postsynaptic components.

The presynaptic component is comparatively simple, in that it consists of depolarisations of the axons, and any presynaptic inhibition. The postsynaptic component is much more complex. It represents the summation of excitation and inhibition. The excitation comprises brief spike action potentials and EPSP's (excitatory postsynaptic potentials). The latter can be quite long in duration and are graded in action (i.e. they can be summated). The EPSP's are due to depolarisations in both the dendrites and the soma of the cell. The inhibition in the postsynaptic component is based on hyperpolarising postsynaptic potentials, called IPSP's (inhibitory postsynaptic potentials). The gross hyperpolarisation effect is a summation of hyperpolarisation due to inhibitory synapses and hyperpolarisation due to after potentials of discharged neurones and fibres.

The earliest theories about the nature of evoked potentials, reviewed by John (1967), suggested that they represented the envelope of discharges in a population of neurones. These envelopes resulted from the summation of spike discharges of individual neurones which had some time dispersion in their firing. Later, Purpura (1959) and Amassian et al (1964) argued that cortical evoked potentials were not based on spike action potentials, but were based on slow potentials such as the postsynaptic and after potentials mentioned above. This evidence was based on experimental dissociation of spike and evoked activity and from implications about volume recording technique (Uttal, 1965). Buchwald et al (1966), who used a multiple unit recording technique, reported that there could be altered unit discharge with no accompanying evoked potential change, and evoked potential change without any concurrent unit activity change. However, close examination of their MG evoked potential records shows a poor signal-to-noise ratio and almost a complete lack of a synchronous evoked response. These results would be more

convincing, and indeed more important, if a synchronous response with good signal-to-noise ratio were associated with the effects.

One problem with the work of Amassian et al (1964) and Purpura (1959) is that it was based on a small sample of spike action potentials (Uttal, 1965). Fox and O'Brien (1965), using computer techniques, have shown relationships that are more in keeping with the old envelope theory. They found marked correlations between the probability of firing of a single cortical cell and an averaged evoked potential waveform. In many cases, they claimed to be able to predict the firing pattern of a cell from the evoked potential waveform, but often the correlation was not very great. The most important feature of these results is that there can be a strong relationship between evoked potential waveform and unit activity. Verzeano et al (1968), recording in the lateral geniculate, found that "the probability of discharge as well as the frequency of discharge of a particular neurone, are greatest at the times which correspond to the deepest negative slope of the gross response". That is, there was a strong correlation between unit firing pattern and aspects of the evoked potential waveform. It is important to point out that these results do not show that <u>all</u> evoked potential waveforms are such that they are highly correlated with single unit histograms. There are other important factors, such as the anatomy of the particular area in relation to "sinks" and "sources" (Chang, 1959; Biedenbach and Freeman, 1964) which also determine the waveform of evoked potentials. But these observations suggest that evoked potential techniques can, in certain circumstances, be an important indication of the mass neuronal activity in a particular area of the brain. They also suggest that Jasper is over-strict in concluding that "it is probably futile to expect to be able to detect electrophysiological correlates of attention or goal directed activation of behaviour by studies of evoked potentials from large populations of neurones" (Jasper, 1963, p.285). It can be granted that microelectrode techniques with their finer grain analysis are potentially more important, but they can be supplemented by evoked potential studies. However, the other extreme position is also difficult to maintain. This is that any amplitude decrement in evoked potentials is a sign of an excitation decrement in the recording area.

All of the experiments discussed in section 1.3 have used the amplitude of auditory evoked potentials as their measure. Thus a decrease in amplitude

is regarded as a sign of habituation. Implicit in all these experiments is the concept that a reduction in the amplitude of evoked potentials is a sign that there has been a reduction in excitation or the net excitatory activity in the particular region. Hernández-Peón (1961), for example, regards such a reduction in the CN response as a decrease in excitation due to inhibition arising in the reticular formation of the brain stem. This line of argument could have been supported if Hernández-Peón's (1956) original work had been confirmed.

Since the CN is the first synaptic area of the auditory pathway, it would be expected that characteristics of the stimulus (such as intensity, time of arrival at the ear) would be securely encoded at this point, and that this would lead to synchronous firing of cells in response to impulsive stimuli. There is evidence of this in a number of single unit studies (Kiang, 1965; Kiang et al, 1965). Thus a reduction of amplitude to noise level would indicate a very drastic excitatory change in this area. However, the results obtained by other workers for this area (section 1.2) reveal amplitude reductions of the order of only 10 - 20 percent. In other auditory areas, when amplitude reductions occur, they are of the order of 20 - 40 percent (section 1.2). It should be pointed out that the higher areas of the auditory pathway show an increasing dispersion of synchronous firing as recordings are made from higher and higher up the pathway (Atkin and Dunlop, 1968). Thus a small reduction in amplitude need not necessarily indicate that there has been a net reduction in excitation, but simply a reduction in synchronous firing.

If reductions in the amplitude of evoked potentials are not necessarily the sign of a net excitation loss, what could they represent? Consider the case where an auditory synaptic area has two classes of hypothetical cells, e.g. P cells, which transmit coded auditory information to the next area, and I cells which are inhibitory interneurones (terminology from Burke and Sefton (1966)). Suppose that continued repetitive stimulation facilitates the excitatory action of the P cells and these in turn increase the activity of the I cells. The macro electrode records a combination of pre- and post-synaptic activity. Let us assume for ease of argument that the presynaptic component is constant, and that the part of the evoked potential contributing to the peak to peak amplitude has a duration of 30 msecs. Since the evoked

potential is a summation of excitation (consisting of unit spikes, dendritic depolarisations and sub-threshold soma depolarisations from both P and I cells) and inhibition (hyperpolarisations from the I cells), it is possible that a reduction of evoked potential amplitude may occur even though there has been a net increase in excitation compared with a single stimulus. For example, the increased amount of hyperpolarisation might show up as an evoked amplitude loss when in fact there is more unit excitation occurring in the P cells. This could occur because the effectiveness of the inhibition might not be a linear function. Thus the increase in hyperpolarisation might not inhibit the spike firing of the same proportion of P cells as the smaller amount of hyperpolarisation.

The above example is rather complicated, depending on assumptions about facilitation of P cells due to repetitive stimulation and non-linearity of inhibitory effects. But it brings out the point that evoked amplitude reductions need not represent net excitation loss. It is also true that they could represent net excitation loss.

There is clearly a major problem concerning the interpretation of amplitude changes in evoked potentials. It is very tempting to dismiss the evoked potential technique entirely as Whitfield (1967) has done. He argues that "such responses tend to be at best uninterpretable and at worst downright misleading in our present state of knowledge of their origin" (Whitfield, 1967, p.2). It is clear that in some situations (Fox and O'Brien, 1965) an interpretation can be given and in other cases an interpretation is difficult to give. However, it is possible to argue that there are at least two more sets of conditions under which an interpretation of excitation loss is reasonable.

The first set of conditions would be if an evoked potential amplitude decrement was related in a systematic way with a stimulus parameter. One example of this would be if the amplitude varied proportionately with stimulus intensity. Another example would be if the amplitude varied as a function of the time since the previous stimulus (i.e. a refractory period situation). While it is logically conceivable that such relationships might represent excitation increases, as the above example indicates, most workers would accept an excitation loss explanation as a working hypothesis.

Another set of conditions would occur if the parameters of AEPH for a given auditory area were isomorphic with the parameters of BRH; in particular, if it were observed that an AEPH decrement was abolished by extraneous stimuli or conditioning procedures. Under these conditions, an explanation in terms of excitation loss would be a reasonable one for evoked potential amplitude decrements. This would, of course, still leave as a separate issue the problem of whether a particular case of BRH is based on AEPH when auditory stimuli are used.

Worden (1967) has raised another problem for the interpretation of amplitude changes in evoked potentials, in that Worden and Marsh (1963) obtained amplitude changes in the opposite direction from adjacent monopolar recordings. Worden has suggested that more stress should be placed on locating electrodes in relation to identified current sources and sinks (Worden, 1967, p.79). Such a procedure has not been attempted in this field (not even by Worden and his co-workers), no doubt because of the difficulty in doing this with chronic animals and large macro electrodes.

However, a close examination of the data of Worden and Marsh (1963) indicates that the evidence for conflicting amplitude variation is far from strong. For example, evidence is presented for monopolar recordings from three cats. Yet in only one cat is there evidence of amplitude changes in opposite directions, and even here, some of the data show changes in the same direction. These results could possibly be explained by acoustic variation, since this was not controlled in the experiment.

It can be concluded that the AEPH situation is one in which it might be possible to interpret changes in evoked potential amplitudes. One interpretation could be based on showing that AEPH has similar relationships to stimulus variables as does BRH. Alternatively, it might be possible to show that AEPH decrements are based on refractory processes. If either of these interpretations were supported, then it would be an important finding for the evoked potential technique, and this possibility indicates that systematic experiments on AEPH are warranted.

CHAPTER 2

2.0 Studies on Methodological Problems

2.1 Introduction

As the above survey indicates, the classical auditory pathway has been extensively studied in recent years by recording from unrestrained, unanaesthetised animals. A number of workers, however, have drawn attention to the need for certain experimental controls to ensure that observed changes are not, in fact, artefacts of uncontrolled variables in the situation (Carmel and Starr, 1964; Marsh, Worden and Hicks, 1962).

2.2 Activation of Middle Ear Muscles

Carmel and Starr (1964) examined the factors that modify middle-ear muscle activity in awake cats. As they pointed out, most studies of this reflex have been concerned with its protective function, such as the protection afforded to the inner ear against damaging intense sounds (Helding, 1961).

Electrodes were placed on the round window and in the middle-ear muscles of cats. The animals were stimulated with white noise of intensity 85 db (re .0002 dynes/cm^2). The most interesting observation for the study of auditory evoked potential habituation is that a number of non-acoustic factors can elicit the middle-ear muscle response. For example, bodily movements are associated with contractions of both the stapedius and the tensor tympani muscles. The extent of these contractions is proportional to the extent of the bodily movement (Carmel and Starr, 1964, p.609). Vocalisation also produces ear muscle activity. These results suggest that, as well as changes in position in an acoustic field, the effects of movements and vocalisations should as far as possible be deleted from records attempting to show auditory habituation. This is not such a difficult task in this type of study since the situation does not require the animals to make any overt response. However, it would seem to be necessary that the animal's behaviour be continuously monitored for movement and vocalisation.

Another important observation noted in this study is that the reflex

contraction of these muscles is very sensitive to barbiturate anaesthesia. This reflex response was no longer present at levels of anaesthesia when other reflexes (corneal and pinna) were still present.

2.3 Variations in the Acoustic Sound Field

Marsh, Worden and Hicks (1962) report that slight changes (as little as 0.5 inch) in the position of an animal resulted in marked changes in both the form and amplitude of evoked potentials recorded in the cochlear nucleus of cats. When the animals (four cats) were stimulated with tone pulses (30 msec duration), then amplitude decrements of up to 50 percent were observed for some changes in position and head orientation. When click stimuli (1 msec duration) were used, then the maximum amplitude change was 37 percent in one cat. Unfortunately, this study does not give information on whether these results occurred in all placements. This is important, because the photographs of oscilloscope traces provided in the paper show only small changes in responses to clicks, but marked changes in form and amplitude of the evoked responses to the tone pulses. These authors also fail to report intensity level of either the clicks or the tones, so that in conjunction with their lack of data on the whole sample, it is difficult to generalise from the result. It is mentioned that the loudspeaker was situated three feet above the floor of the training box which was 16 inches high. This must mean that the loudspeaker was outside the box. This seems in keeping with their description of a similar box in another paper (Worden and Marsh, 1963). It could be that this type of positioning of a loudspeaker would enhance sound field variation more than an enclosed box.

One implication from this study is that the conditions of this test box could explain the varied results obtained in their earlier study (Worden and Marsh, 1963). For example, one cochlear nucleus placement showed amplitude decrements of 20 percent on one run and increments of ten percent on the next run.

In another study, Worden, Marsh, Abraham and Whittlesey (1964) compared the variation in evoked potentials recorded at the CN, IC and the auditory cortex under two conditions of stimulus presentation. One used the loudspeaker system reported above and the other used small earphones

attached to the cat's head by ear moulds. The stimuli were tone pulses of 30 msec duration and of 60 - 92 db intensity. They found more variability with the loudspeaker; this variability was much reduced as the recordings were made higher up the auditory pathway. They suggested that the higher centres are "primarily influenced by central variables not directly related to characteristics of the stimulus" (p.530). This does not necessarily follow from their results, since one simple explanation could be that as the higher centres have binaural input they would be less affected by a change at one ear.

Simmons and Beatty (1964) have presented evidence that the amplitude of the cochlear microphonic response shows variability when an anaesthetised animal's head is moved in a continuous sound field. They argued that these differences are due to "head shadows and to peaks and nulls in the standing wave pattern" (p.333). In their experimental situation, the loudspeaker was also outside the recording cage.

Thus it would seem that, unless some evidence for controlling acoustic variability is available, any study of AEPH would be suspect. Changes in amplitude due to acoustic variation could be confounded with habituation decrements. It would seem that this confounding could be reduced by two methods. One is to use loudspeakers and to devise a situation in which variability is kept to a minimum. In conjunction with this, some sort of spatial criteria would have to be set for movement and position of an animal's head in the acoustic field. The second method would be to put small earphones on the head of the animal (as reported above) and control possible artefacts directly.

2.4 Experiment 1 - Acoustic Variation in Test Box

2.4.1 Introduction

The aim of this experiment was to evaluate the acoustic properties of an enclosed box which was to be used for studies of AEPH.

2.4.2 Apparatus

The test box was a completely enclosed box with internal measurements of 22 inches in all dimensions. Attenuation of external sound sources was achieved by packing the three-inch space between double walls above a height of three cm with sound absorbent material (Insulwool). Three layers of one-way glass, each separated by a space of one inch, covered the lower three inches around the four walls of the box to allow observation of the subject. The interior of the box above the three cm level was lined with tempered masonite and the front opening of the box was enclosed with a tightly fitting and overlapping lid constructed in the same way as the walls. Two five-inch loudspeakers were located centrally in opposing walls of the cage (see figs 2-1 and 2-2). The cage was placed on a heavy wooden table which was mounted on rubber blocks to reduce sound transmission through the floor of the laboratory.

2.4.3 Stimulus Parameters

It was intended to employ impulsive stimuli with short rise times because most studies in this area had employed such stimuli, and impulsive stimuli with short rise time assure synchronous firing at peripheral areas of the auditory pathway (Goldstein and Kiang, 1958). Two groups of stimuli were generated by Tektronix waveform and pulse generators. One group consisted of clicks of 20 msec duration comprising a burst of pulses at a frequency of 1,000 per second. The other group consisted of clicks of 0.2 msec duration. Both groups of stimuli had a rise time of ten microseconds (measured on an oscilloscope). The output of the waveform and pulse generators was fed to a voltage amplifier with a calibrated ten turn potentiometer. This allowed a considerable increase in voltage to drive the transducers.

Figure 2-1

A photograph of the test box showing the overlapping door and the one-way glass. The box is on a layer of Insulwool which is on a heavy table mounted on rubber blocks. Next to the box can be seen four Tektronix 122 amplifiers and the Offner EEG system.

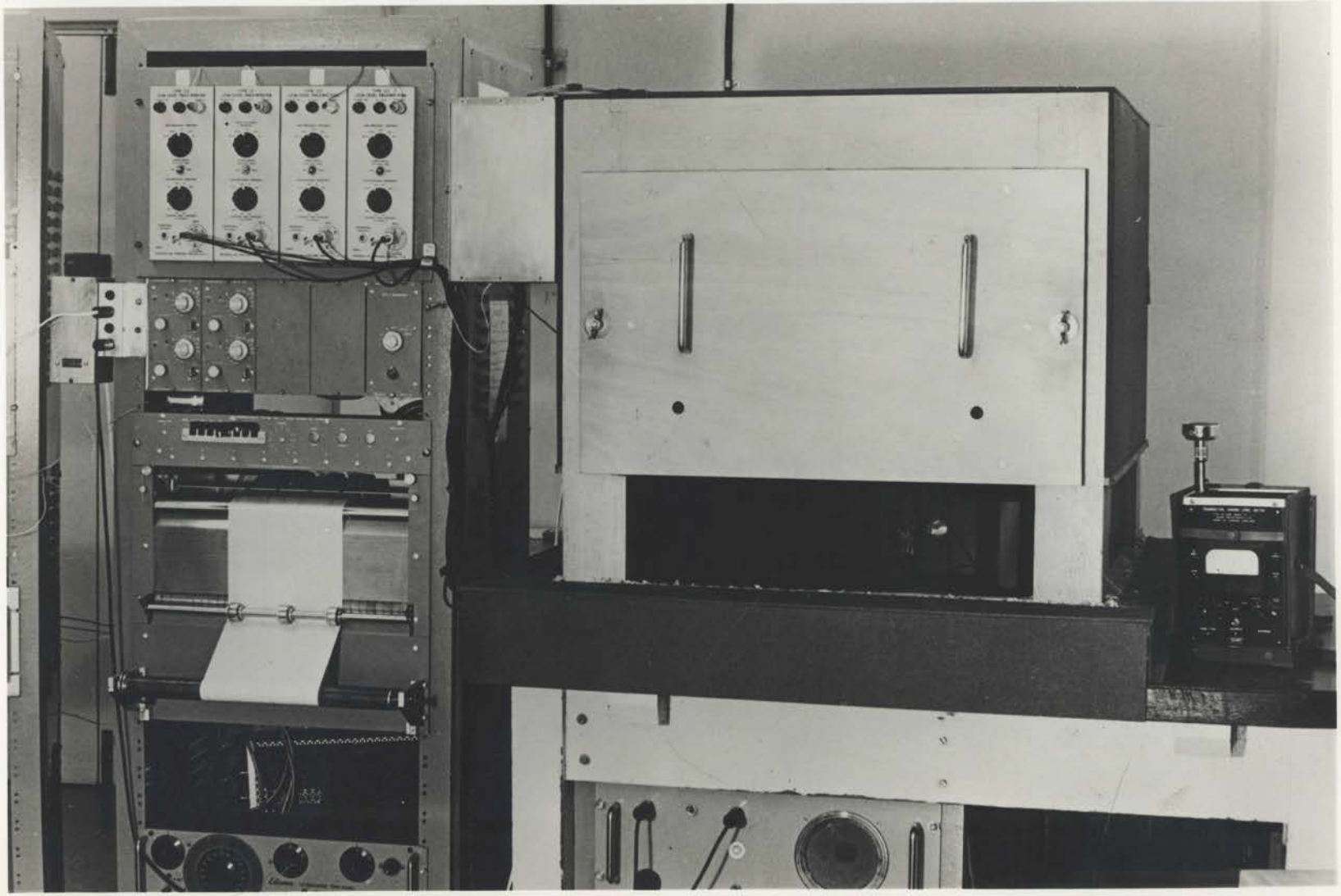
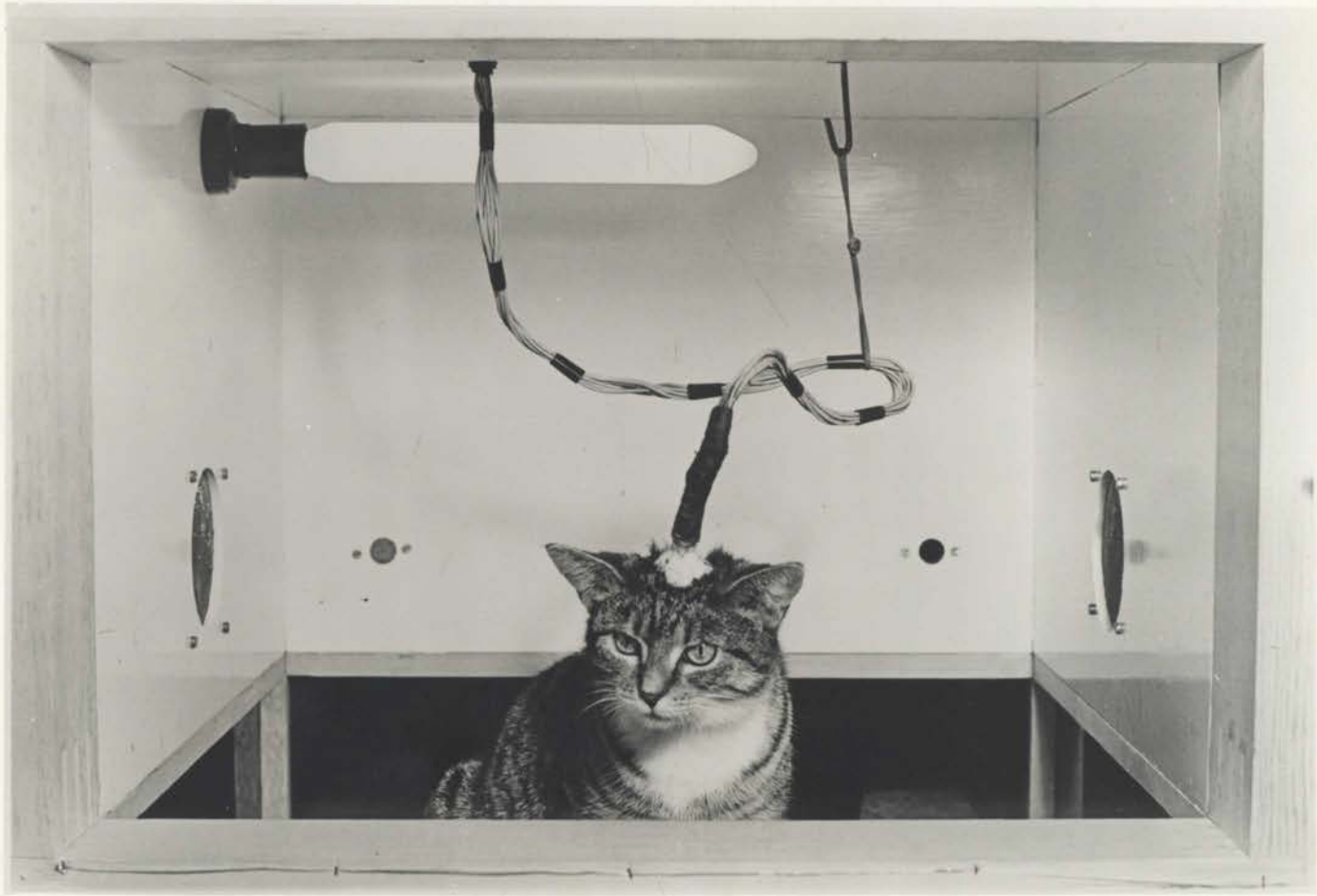


Figure 2-2

A photograph of the interior of the test box showing the two loudspeakers and the coaxial cable inserted into a socket mounted on an animal's head.



2.4.3.1 Stimulus Intensity

There are problems in measuring the intensity of impulsive auditory stimuli with short rise times. This cannot be done with the conventional sound level meter, since the inertia in such an instrument will not allow an accurate measurement of peak sound pressure level. There are two major techniques for making such measurements. One is to calibrate an oscilloscope which receives the output of the amplifier of a sound level meter. This will allow measurements of the peak sound pressure level re .0002 microbar. The other technique is to use a commercial impact noise analyser, with a condenser system capable of storing and recording peak sound pressure levels. In most of the studies reported in Chapter 1 it appears that these techniques were not employed. In this and all the later studies, the decibel readings reported are peak sound-pressure levels measured by a General Radio impact noise analyser (type 1556-A) in conjunction with a General Radio sound level meter (type 1551-B).

In this study the intensities of the 20 msec clicks employed were 85 and 105 db re .0002 microbar and the 0.2 msec clicks were 80 and 100 db re .0002 microbar. These might appear to be excessively high intensities to workers accustomed to measuring clicks with only a sound level meter, but the latter procedure would produce values of 20 - 25 db lower than these peak values.

2.4.4 Subjects and Chronic Electrode Implantation Technique

Bipolar stainless steel electrodes were implanted in either the left or right cochlear nucleus of five cats. The bipolar electrodes were prepared by baking two pre-enamelled stainless steel wires (125 microns in diameter) on to a central strut (700 microns in diameter) with epoxylite enamel. The electrodes were insulated except at the recording tips which were bared for one mm. There was a one mm gap between the tips.

The electrodes were implanted at Horsley-Clarke co-ordinates using a Trent Wells stereotaxic instrument. The general technique is similar to that described by Jasper and Ajmone-Marsan (1960).

Healthy cats were selected and anaesthetised with intraperitoneal injections of Pentobarbital (45 mg/Kg - Nembutal, Abbott). Each animal was

examined with an aurioscope before surgery and only animals without middle-ear infection were used.

Each animal was then placed in the stereotaxic apparatus. The scalp and part of the neck were shaved. The skin was incised on the midline from the external occipital protuberance to the supra-orbital ridge. Then the flaps of skin were retracted, underlying fascia were removed, and the temporalis muscles reflected as far laterally as possible. The exposed skull was then thoroughly cleaned of blood and muscle. The skull was marked at the point of entry of the electrode and a five mm hole drilled in the skull with a dental burr. A stainless steel screw was placed in the skull close to each hole so that a support would be available on which dental cement could grip. The dura mater below each hole was incised with a suture needle and any bleeding stopped by the application of "Sterispon" absorbent foam.

In each cat the cochlear nucleus electrode was located at P7.5, L-R 8.0, H-5.5 according to the stereotaxic atlas of Snider and Niemer (1961). To approach the CN, the electrodes were angled at 55° to the vertical so that they passed under the bony tentorium in a ventral-anterior direction. Prior trigonometrical calculations had shown that this orientation would place the tips in the required co-ordinates. Throughout the placement of each electrode, auditory stimuli were delivered through hollow ear bars on the stereotaxic instrument. This monitoring procedure allowed measurement of latency and confirmation of the placement in the auditory pathway. Only responses having latencies of approximately 1.5 - 2.0 msecs (Ades and Brookhart, 1950) were accepted as being recorded in the CN.

When the electrode was in position, bone wax was carefully packed around and in the electrode hole. This was done for three reasons:

(1) To prevent dental cement touching the cortex, since it is toxic to brain matter,

(2) To prevent the electrode from moving,

(3) To prevent fluid seeping onto the skull.

When this was completed and the skull quite dry, dental cement (TEKTON) was poured around the electrode and adjacent screw. The cement was allowed to harden. Then the electrode holder was removed and the central strut cut

off. More dental cement was added to cover the electrode, leaving the two electrode wires protruding.

All electrode wires were soldered onto the terminals of a Winchester socket (m9s). This was then located on the skull just behind the frontal sinuses. Four to six additional screws were inserted into the skull around the socket. These were intended to give the dental cement a firmer grip. One of these screws had a length of the pre-enamelled stainless steel wire soldered to it. This was soldered to the socket and acted as a reference lead for all recordings. All of these were covered with dental cement and only the top portion of the socket was exposed.

The muscles were placed back into position and the inside of the wound treated with a topical antibiotic powder (Neosporin). The skin flaps were stitched together using interrupted sutures and further antibiotic powder was applied. Animals were given daily intra-muscular injections of Penicillin-G (300,000 units/day) for the first five post-operative days. Two to three weeks were allowed for post-operative recovery.

2.4.5 Histological Verification of Electrode Placements

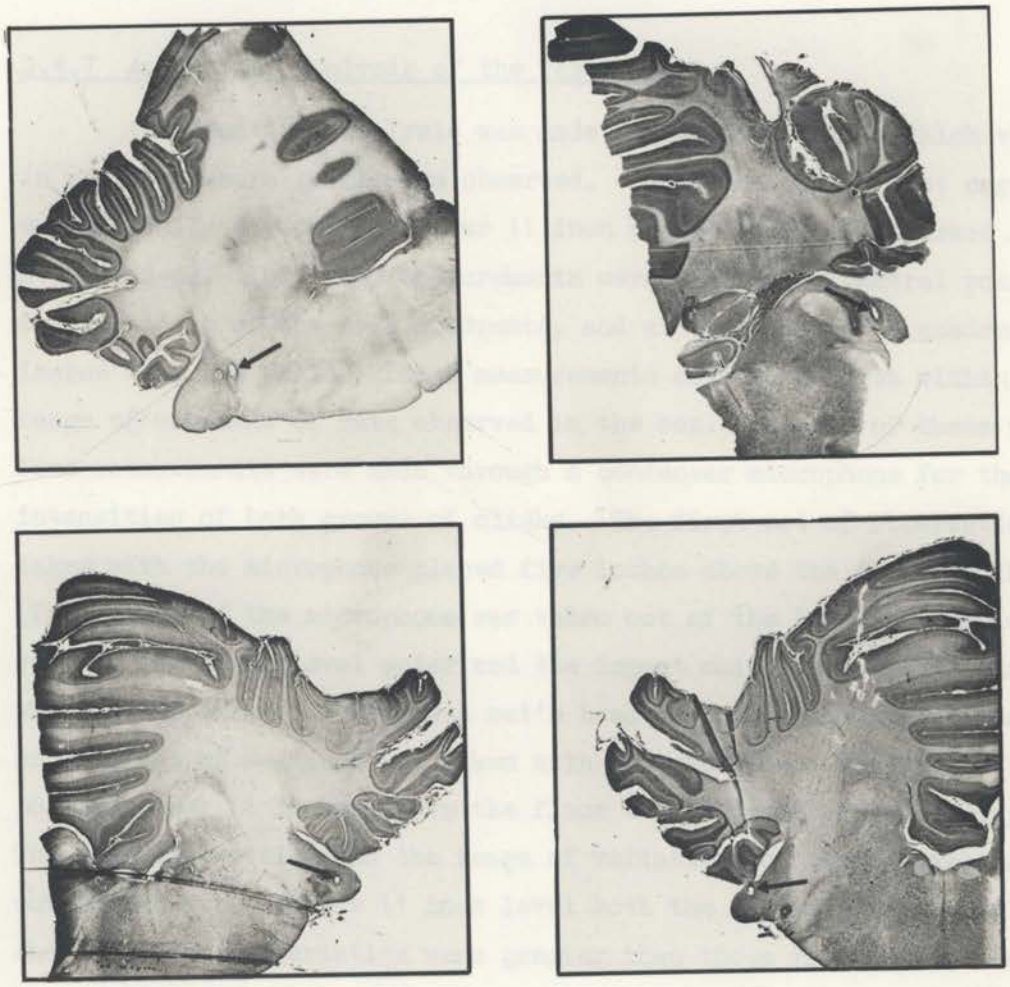
At the conclusion of any set of experiments for a group of animals, iron was electrolytically deposited from the electrode tips. The animals were given an overdose of barbiturate anaesthetic and perfused via the left ventricle of the heart with one percent potassium ferricyanide in 0.9 percent saline (Prussian Blue Reaction). This was followed by ten percent Formalin. The animal's head was removed and placed in ten percent Formalin for two weeks to harden. At this stage, the skull was opened and the brain removed, washed with water, dehydrated with alcohol, embedded in colloidin and serially sectioned (30 microns thickness). Selected sections were mounted and counterstained with cresyl violet. The location of electrode tips was then verified. Figure 2-3 shows the approximate location of the electrodes in four cats.

2.4.6 The Recording System

Each animal was tested in a sound attenuated, electrically shielded test box (see 2.4.7). A Winchester plug (m9p) was inserted in the socket

Figure 2-3

Photographs of serial sections through the dorsal cochlear nucleus for four of the cats in Experiment I. Arrowheads show the location of lesions made through the electrode tips.



in the animal's head (see Figure 2-2). The socket was connected to a miniature coaxial cable which led via a junction box to Tektronix 122 low level pre-amplifiers with band pass settings of 1Kc - 8cps. The pre-amplifiers were coupled to the power amplifiers of an Offner Type R Dynograph Electroencephalographic System. The responses were also displayed on a Tektronix RM-561 oscilloscope. This oscilloscope had an RM-561 slave oscilloscope to which was attached a Grass Kymograph camera.

2.4.7 Acoustical Analysis of the Test Cage

An acoustical analysis was made of the test box, in which variations in sound pressure level were observed. The floor of the test cage was schematically divided into four 11 inch square quadrants, marked A, B, C and D (Fig. 2-4). Intensity measurements were taken at a central position, at the mid-point of the four quadrants, and at the corners of quadrants 3.5 inches from the walls. These measurements covered an area within the normal range of movement of cats observed in the box. At each of these positions, five measurements were made through a condenser microphone for the two intensities of both groups of clicks. The first set of observations was taken with the microphone placed five inches above the floor of the cage. (The output of the microphone was taken out of the box via a cable in the roof to the sound level meter and the impact noise analyser.) This height was the approximate level of a cat's head when in a sitting relaxed position. Another set of readings was taken with the microphone in the same horizontal position, but 11 inches above the floor at the level of the loudspeakers. The mean intensities and the range of variation for these positions are set out in Table 1. At the 11 inch level both the absolute intensity (4-7 db) and the range of variation were greater than those recorded at the five inch level. The greater differences between vertical levels, compared with the variation at a given level, suggests that the important variable to control would be movement in the vertical dimension.

TABLE 1

Microphone height (inches above floor)	Stimulus	Peak Sound Pressure Level (db re .0002 microbar)
5	Click	80 ± 3
5	Click	100 ± 3.75
5	Pulse burst	85 ± 1.5
5	Pulse burst	105 ± 1.5
11	Click	87 ± 5
11	Click	106 ± 3
11	Pulse burst	90 ± 2.5
11	Pulse burst	109 ± 4

2.4.8 The Influence of Cat's Position in the Box on CN Responses

The aim of this section of the experiment was to determine the influence of position on the amplitude and form of evoked responses recorded from the CN of the five cats. As the cats were tested in an unanaesthetised, awake condition, it was not possible to localise each of them in an identical position. As a consequence the criterion adopted was one of a particular area and the schematic quadrants were used. Each animal had been given a number of familiarisation trials in the test box over a period of weeks before testing commenced. They became accustomed to remaining in a small metal tray containing sawdust and would often remain in this area for some hours on end. It became possible to shift each cat into the various quadrants by simply moving it and the small tray.

After noting the position of the head, which was always within the range of four to six inches above the floor at the level of the interaural plane, testing was commenced. The order of presentation of stimuli and the order of quadrants tested was randomised over cats. Upon completion of the measurements for one quadrant, the animal was shifted into another quadrant and once again allowed to settle into position. The procedure was continued until recordings were made in each quadrant. One cat, however, refused to stay in quadrant D and complete records were only obtained for quadrants A, B and C.

2.4.9 Results

The peak-to-peak amplitude of six superimposed evoked potentials was obtained from each cat under each stimulus condition in the quadrants A, B and C. The mean amplitudes of the responses for the five cats are set out in Table 2. For a given intensity, the response amplitude variation over quadrants did not exceed 13 percent for 0.2 msec clicks and 12 percent for 20 msec click bursts compared with the centre position. The means for the clicks and click bursts were analysed in two separate analyses of variance (McNemar, 1959, pp.317-325), summarised in Table 3. There is no significant effect for either stimulus attributable to quadrants, but both clicks and click bursts produced a significant effect ($p < .01$). There is a significant effect due to subjects, but this is simply a statement of individual differences. There is also a significant subjects times intensity interaction which indicates that the form of the relationship between evoked potential amplitude and intensity varies from subject to subject. In absolute microvolt terms, the variation in evoked response amplitude appears commensurate with the range of intensity variation reported in Table 1. The records were also scrutinised for variation in the form of the evoked potentials. The CN potentials obtained from any one animal were relatively constant in form from quadrant to quadrant for both groups of stimuli. Figure 2-4 shows responses for one cat and these observations are representative for all five cats. It is interesting to note that the sort of variation shown in Figure 2-4 is very similar to the variation in form and amplitude shown in the published records of Marsh, Worden and Hicks (1962) for click stimuli.

2.4.10 The Influence of Position on Cochlear Responses

One cat was anaesthetised with Nembutal (60 mg/Kg) administered intraperitoneally. Supplementary injections were given as required. The bulla on one side was exposed and a small hole drilled in its wall allowing a monopolar stainless steel electrode (150 microns in diameter) to be placed adjacent to the round window. The electrode was then fixed to the bulla with dental cement. A reference electrode was placed on the neck muscles adjacent to the bulla. This preparation was used to record cochlear microphonic and auditory nerve responses.

This anaesthetised preparation was placed in positions simulating some of those observed with the awake cats and responses were recorded to both

TABLE 2

Stimulus	Quadrant A	B	C
Pulse burst (85 db)	219.6	220.4	223.6
Click (80 db)	228.0	243.0	227.2
Pulse burst (105 db)	346.4	339.0	342.4
Click (100 db)	316.6	290.4	280.0

TABLE 3

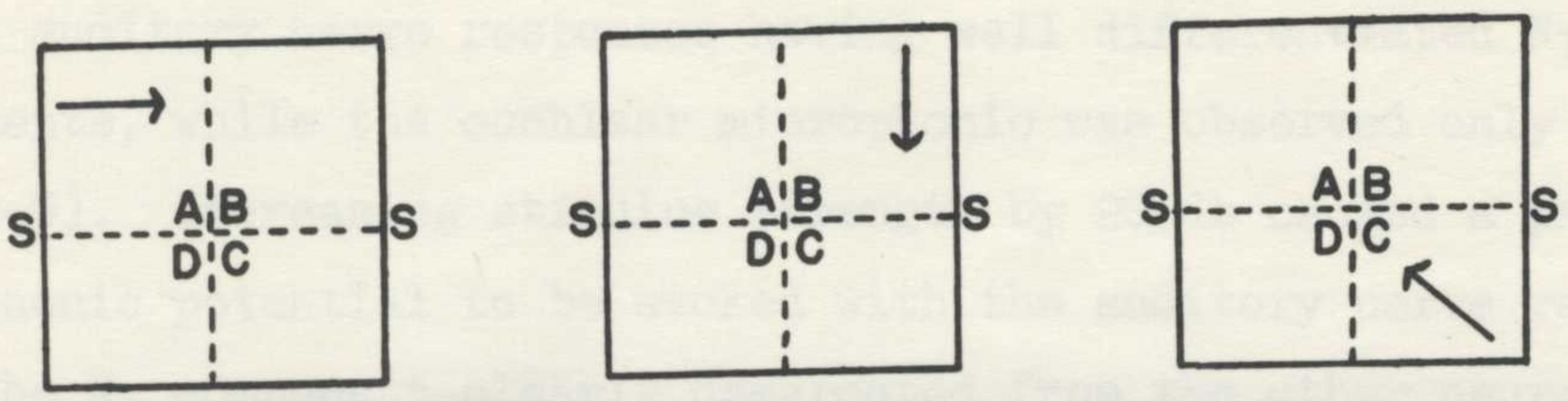
SOURCE	SS	DF	MS	F
(a) Summary of Analysis of Variance for Click Stimuli				
Intensity (I)	26,284.8	1	26,284.8	28.19 **
Position (P)	1,344.3	2	672.2	.72
Subjects (S)	891,244.5	4	22,822.2	239.00 **
I x P	1,632.8	2	816.4	.88
I x S	16,844.6	4	4,211.1	4.52 *
P x S	1,892.4	8	236.6	.25
I x P x S	7,458.5	8	932.3	
TOTAL	946,701.9	29		
(b) Summary of Analysis of Variance for Pulse Burst Stimuli				
Intensity (I)	48,977.5	1	48,977.5	15.78 **
Position (P)	53.6	2	26.8	.07
Subjects (S)	484,423.2	4	121,105.8	390.16 **
I x P	44.0	2	22.0	.07
I x S	26,013.2	4	6,504.4	20.95 **
P x S	1,096.4	8	137.1	.44
I x P x S	2,483.3	8	310.4	
TOTAL	563,091.2	29		

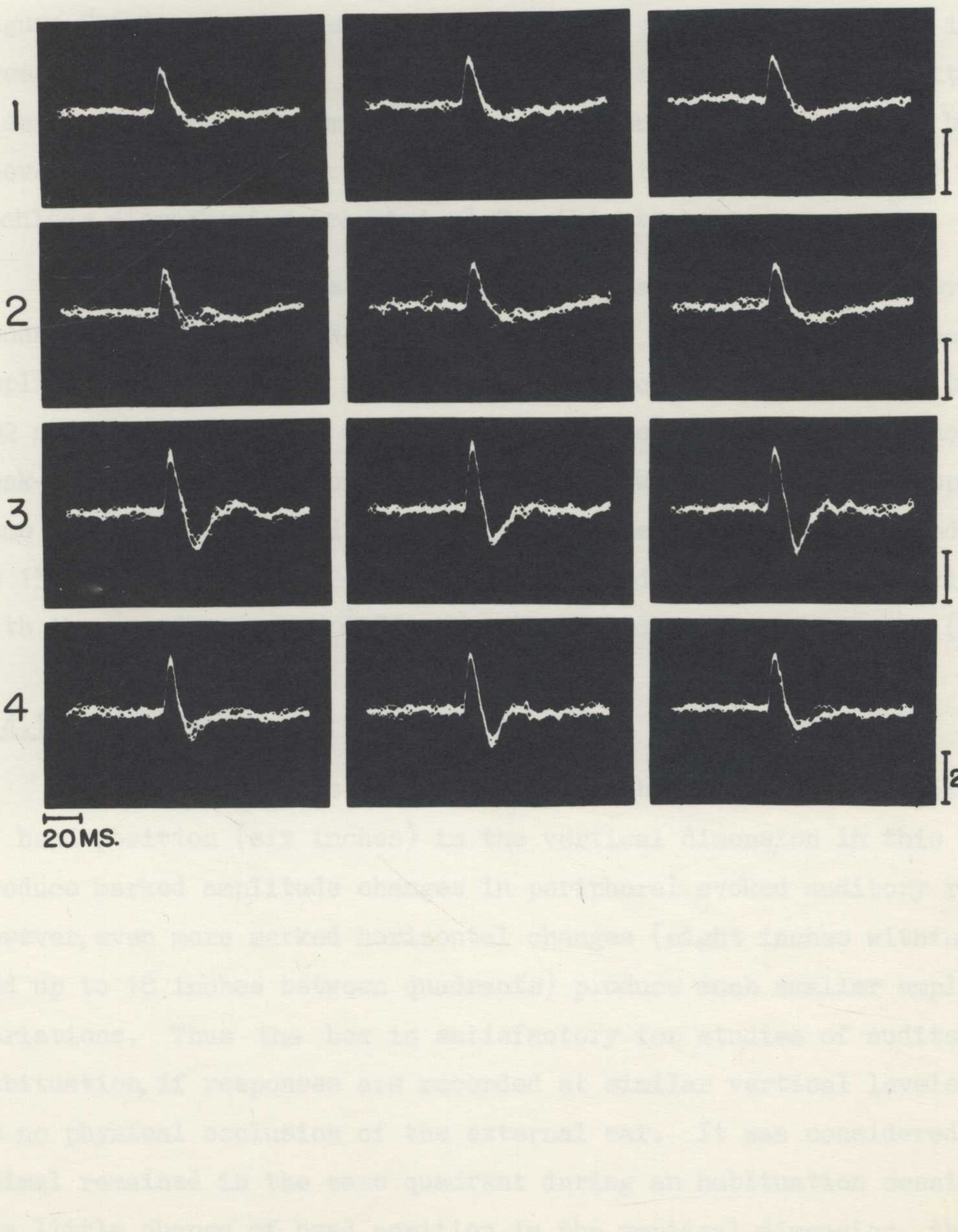
** significant at .01 level

* significant at .05 level

Figure 2-4

Recordings of CN responses taken in various positions in the test box. Each record contains five superimposed responses. Rows 1 and 3 show responses to 80 and 100 db 0.2 msec clicks. Rows 2 and 4 show responses to 85 and 105 db 20 msec clicks. The arrowhead in the figures above each column show the position of the animal's head when the recordings in that column were obtained.


S
A
B
D
C
S
S
A
B
D
C
S
S
A
B
D
C
S


1
2
3
4
200μV
20MS.

groups of stimuli. The same recording techniques were used as described in section 2.4.5.

2.4.11 Results

To the less intense stimuli (80 - 85 db) the round window response showed auditory nerve responses having well differentiated N_1 and N_2 components, while the cochlear microphonic was observed only with difficulty (Fig.2-5). Increasing stimulus strength by 20 db caused a prominent microphonic potential to be evoked with the auditory nerve response having only the N_1 component clearly demarcated from the other neural activity. In Figure 2-6 it can be seen that the type of variation at the CN is also present at the level of the cochlea. Allowing for the difficulties in disentangling neural components from the cochlear microphonics by the above techniques, it can be seen that variations in both the N_1 and the cochlear microphonics are minimal for this test box.

When the cat's head was elevated to a position at the level of the loudspeakers, then the N_1 and N_2 components showed marked increases in amplitude over the five inch level. A typical response evoked by an 80 db .02 msec click stimulus in quadrant A at the five inch level, showed an N_1 peak-to-peak amplitude of 250 microvolts. When the head was kept in the same position but raised vertically, then the amplitude increased by as much as 150 microvolts (Fig.2-7). These amplitude changes agree satisfactorily with the sound pressure differences recorded at the two levels (Table 1).

2.4.12 Discussion

From the two sets of results it can be concluded that marked changes of head position (six inches) in the vertical dimension in this test cage produce marked amplitude changes in peripheral evoked auditory responses. However, even more marked horizontal changes (eight inches within a quadrant and up to 18 inches between quadrants) produce much smaller amplitude variations. Thus the box is satisfactory for studies of auditory evoked habituation, if responses are recorded at similar vertical levels and there is no physical occlusion of the external ear. It was considered that if an animal remained in the same quadrant during an habituation session and there was little change of head position in the vertical dimension, then these criteria would be adequate.

Figure 2-5

Round window responses to clicks of varying intensity and duration recorded in three positions in the test box. The responses in Row 1 were evoked by 20 msec, 85 db clicks, in Row 2 by 80 db, 0.2 msec clicks, in Row 3 by 20 msec, 105 db clicks, and in Row 4 by 100 db, 0.2 msec clicks. The responses in each column were taken in the same position in either quadrant A, B or C.

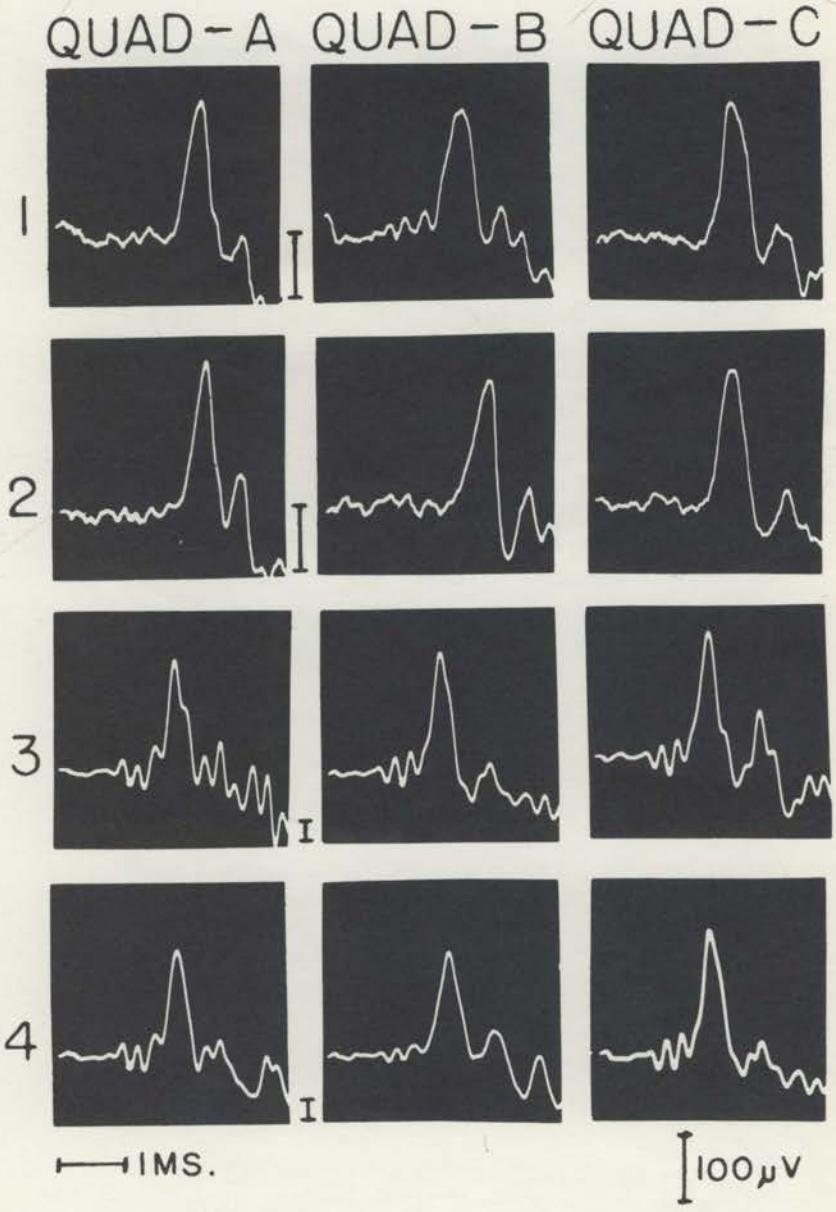
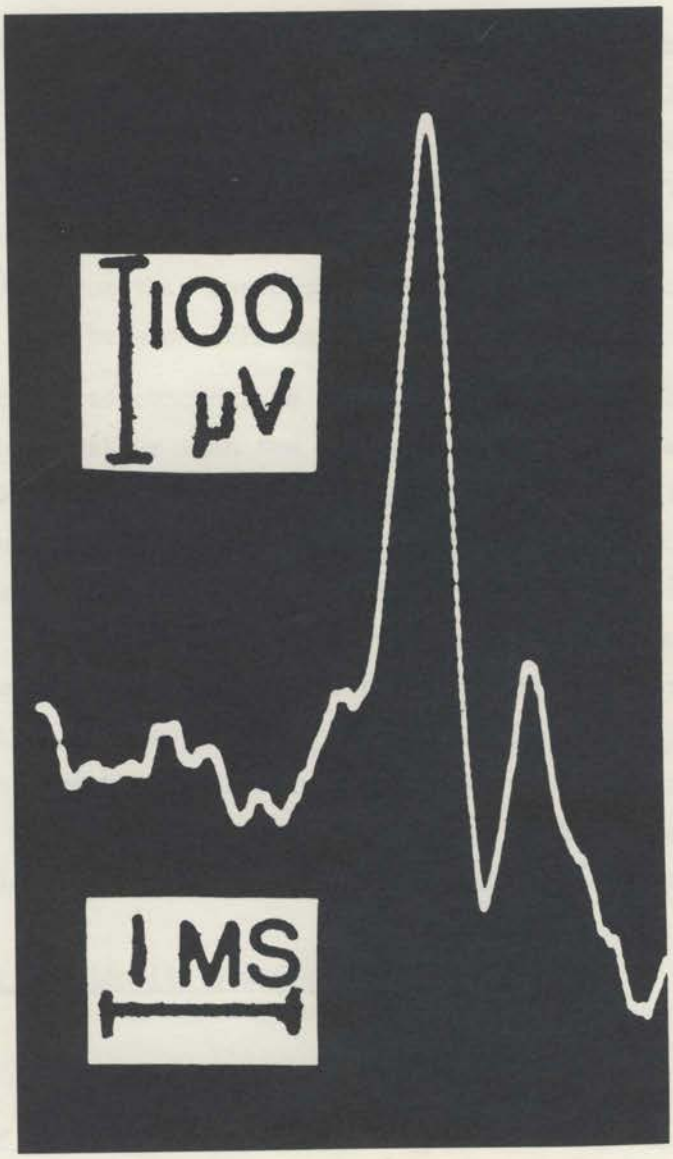

QUAD-A
QUAD-B
QUAD-C
1
2
3
4
1MS.
100μV

Figure 2-6

Round window recording taken with the cat's head placed on the same level as the loudspeakers in the test box. The stimulus was an 80 db click of 0.02 msec duration.


100
μV
1 MS

CHAPTER 3

3.0 Parametric Studies of AEPH

3.1 Introduction

The aim of the experiments in this section was to establish the reality of the phenomenon of auditory evoked potential habituation (AEPH) and to study systematically the effects of a number of stimulus variables on its development. In particular, a number of stimulus factors will be examined which have been shown to be important parameters of behavioural response habituation (BRH). From this approach, it may be possible to determine the relationship, if any, between AEPH and BRH.

It was decided to concentrate the experiments on subcortical areas of the auditory pathway rather than the auditory cortex. Before the advent of recording from chronic animals it had been assumed that BRH in its more complex forms was mediated by the cortex. For example, Pavlov (1941) reported that the orienting reflex did not habituate in decerebrate dogs as it did in normal controls. Thus some of the early studies, summarised in Chapter 1, showing subcortical AEPH were of great interest both to physiologists and to psychologists (Bruner, 1956). Consequently, it was felt that a systematic analysis of habituation in subcortical areas would be of prime importance. The CN was chosen since the original work in the field had been in this nucleus. The IC and the MG were also selected for study.

3.2 Experiment 2 - Intensity Studies Using Loudspeakers

3.2.1 Aim

The aim of this experiment was to vary stimulus intensity systematically, so as to determine the relationship between stimulus intensity and evoked response amplitude changes recorded in the CN, IC and MG during repetitive auditory stimulation. It was also decided to use a stimulus that was as similar as possible to the one used by Hernández-Peón et al (1955). It was felt that this would allow a more adequate test of some of his findings.

3.2.2 Subjects and Electrode Implantation

Bipolar stainless steel electrodes were implanted in ten cats in the MG (A5.0, L11.0, H0.0), the IC (P1.5, R5.0, H2.0) and the CN (P7.5, R8.0, H5.5) according to the stereotaxic atlas of Snider and Niemer (1961). The general procedure and type of electrode were similar to that described in section 2.4.4. In addition, only responses having latencies of approximately 6, 3, and 1.5 - 2.0 msec (Ades and Brookhart, 1950) were accepted as being recorded in the MG, IC and CN, respectively. From this population of cats, five placements were selected from each area which yielded evoked auditory potentials with a signal-to-noise ratio of at least two to one. It proved to be most difficult to meet these criteria in the MG. Often a response was obtained during implantation that was suitable by the criteria of amplitude and latency, but later the placement had to be discarded because of poor signal-to-noise ratio or complete loss of evoked activity. The same difficulty has been reported by other investigators (Ades and Brookhart, 1950; Kemp, Coppee and Robinson, 1937). Extremely slow electrode penetration seemed partly to overcome this problem. It would seem that this area is susceptible to damage by the electrodes being moved too quickly through the area. Two to three weeks were always allowed for recovery from the operation before testing began.

3.2.3 Stimulus Parameters

The auditory stimulus consisted of a train of 0.1 msec square wave pulses presented for a duration of 20 msec at a rate of 1,000 per sec. This stimulus will be referred to as a 20 msec click throughout. The click was generated by Tektronix waveform and pulse generators (see section 2.4.3). Four stimulus intensities were selected. They were peak sound-pressure levels of 75, 85, 95 and 105 db re .0002 microbar. These measurements were obtained using a General Radio impact noise analyser (type 1556-A) and sound level meter (type 1551-B) in a similar manner to the methods reported in section 2.4.3.1. The stimulus repetition rate was 1/2 sec. This stimulus duration and repetition rate is the same as that employed by Hernández-Peón et al (1955).

3.2.4 Test Environment, Design and Data Collection

The test cage used in this study was the one described in section

2.4.2. Before being tested, each cat was placed several times in the cage for periods of one to two hours to accustom it to the experimental situation.

Bipolar recordings were obtained from the unanaesthetised cats via a shielded cable (Microdot coaxial) connected to Tektronix 122 preamplifiers and from there to an Offner EEG system (section 2.4.6).

Each animal was stimulated by a click every two sec for a period of 95 minutes. To cope with the problem of movement in the acoustic field, it was necessary to run some animals twice at each intensity level. To keep the number of session equal all animals had two runs. The order of stimulus intensity presentation was randomised over cats and the first successful (i.e. by the spatial criteria of remaining in the one quadrant) run was accepted as the data for that intensity. At least seven days were allowed to elapse between consecutive sessions for any one cat. The evoked responses were recorded for the first five minutes and during every alternate five minutes thereafter.

3.2.5 Data Reduction

The peak-to-peak deflection of each evoked response was measured by ruler to the nearest millimetre and this score was transformed to microvolts.

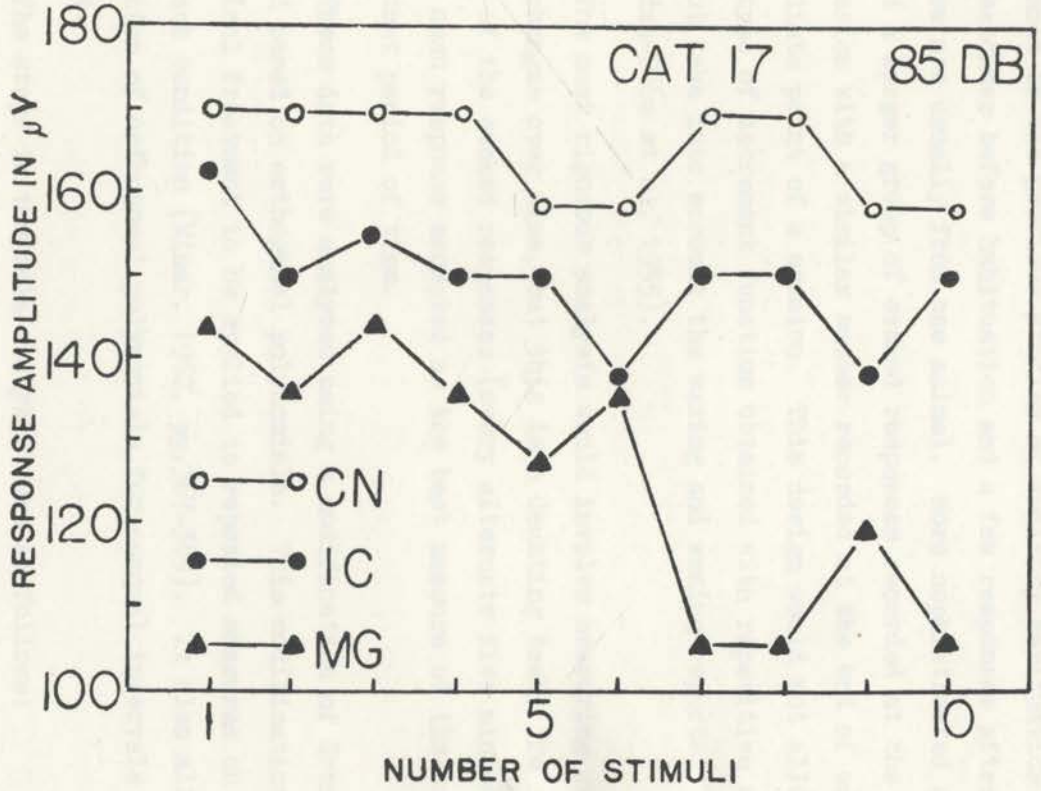
Evoked potentials that were influenced by transient movements and vocalisation artefacts were discarded by omitting a group of potentials around the suspected responses.

It was decided to select the first four responses as the control level. Preliminary work (see Fig.3-1) indicated that the MG could show reductions in the first ten responses. Thus the type of control level used by Marsh et al (1961) of their first fifty responses could mask the size of, or even the actual presence of, a decrement. In a number of preliminary sessions before testing commenced, a few isolated stimuli were given to establish gain settings for each placement and to check latency and amplitude criteria. The results of the first four responses approximated more closely these figures than the first 30 or the first 50 responses of a session.

The mean response amplitude was calculated for each five minute period of recording. This mean figure was then expressed as a percentage of the

Figure 3-1

The amplitude in microvolts of the first ten responses of one cat in an habituation session recorded at the CN, IC and MG. Stimuli were presented at the rate of 1/2 sec.


CAT 17
85 DB
180
160
140
120
100
RESPONSE AMPLITUDE IN μV
CN
IC
MG
1
5
10
NUMBER OF STIMULI

control value. Thus two sets of data were obtained: (a) peak-to-peak amplitude in microvolts, (b) this amplitude expressed as a percentage of the control microvolt amplitude.

3.2.6 Statistical Analysis

Most of the previous studies of auditory habituation have shown only a few responses before habituation and a few responses after habituation, and these are usually from one animal. More sophisticated experimenters have compared a larger group of evoked responses recorded at the beginning of a test session with a similar number recorded at the end of or at some intermediate point of a session. This design would not allow any indication of the type of decrement function obtained with repetitive stimulation and would not take into account the waxing and waning reported by some workers (Hernández-Peón et al, 1955).

The most rigorous analysis would involve measuring every response and noting changes over time, but this is a daunting task. To reduce labour, samples of the evoked responses (every alternate five minutes) were measured, and the mean response accepted as the best measure of the true score for a cat at that period of time.

These data were analysed using a modification of Grant's (1956) test of trend based on orthogonal polynomials. This modification allows statistical treatment to be applied to repeated measures on the same subjects under each condition (Winer, 1962, pp.367-369). It also allowed the calculation of orthogonal polynomials for unequal intervals of the independent variable.

The steps in the data analysis were as follows:

(1) the mean data were scaled (e.g. 90 μv = .90 or 90 % = .90) and punched onto IBM cards for analysis by an IBM 1620 computer.

(2) Both sets of data (microvolt and percentage) were tested for independence of mean and variance. This analysis consisted of the determination of the correlations between means and the standard deviations, the means and the variances and the square of the means and the standard deviations at each point on the abscissa (time) (Maxwell, 1958, p.73). The microvolt data showed a high correlation between the means and the standard deviations, indicating

that a logarithmic transformation would be suitable. An iterative program was designed to perform successive logarithmic transformations of data until the correlation between the means and the standard deviations was close to zero. This particular transformation was then accepted for further analysis.

(3) The microvolt data, the transformed microvolt data and the percentage data were analysed separately using the modified test of trend program.

The third step could be regarded as analysing the same data in a number of ways and thus being invalid. However, the method was adopted for purely empirical reasons. Firstly, there is evidence that the F and t statistics are robust with respect to violations of the assumption of normality of population distributions and homogeneity of variance, provided that sample sizes are equal and 2-tailed tests used (Boneau, 1961). But this evidence applies only to a two group situation, that is, a simple design. There is no evidence available about the influence of homogeneity of variance on complex designs such as Grant's test of trend. However, tests of homogeneity of variance are highly sensitive to departures from normality (Rodger, 1966). This has lead one author (Box, 1953) to claim that using these tests is "like putting to sea in a rowing boat (homogeneity test) to find out whether it is calm enough for a liner to sail (analysis of variance)" (quoted by Rodger, 1966, pp.22-23).

Maxwell (1958) points out that it is often found that some data showing a non-significant result with a test for homogeneity will still have, for example, a clear relationship between means and standard deviations. He argues that "it is always desirable before performing an analysis of variance to inspect the data and perform a suitable transformation to render the means and the variances independent if they are found not to be so ... these transformations, apart from stabilising the variance for changes in the mean, have an additional advantage in that they tend to make the data more normal. When required they should be carried out before tests of homogeneity of variance are considered; indeed they obviate the need for the latter" (Maxwell, 1958, pp.72-73). For these reasons, it was decided to use an empirical approach based on Maxwell's (1958) method. When a correlation was found between the means and the standard deviations,

the data were transformed and analysed to see whether this complex design was also robust. The results show that the analyses of transformed and microvolt data yielded similar results as the non transformed data, in that the same components are significant in both cases. Therefore it appears that this test could be fairly robust to violations of this assumption.

However, it was decided to use only the percentage data for further analysis. The reason for this is that the test of trend allows a statement about the difference between the means of each condition apart from the differences between trend components such as linear, quadratic etc. But the microvolt data already have a difference between means prior to any testing because a more intense stimulus tends to evoke a larger response amplitude. Thus any difference between means due to stimulation over time could be confounded with differences due to stimulus intensity per se. To overcome this, a percentage transformation has been used which gives each group a similar control level. It would be more elegant to use some sort of covariance design but this has not been developed for complex tests of trend.

A major difficulty in the statistical treatment of complex designs is the handling of multiple contrasts (Cochran and Cox, 1957; Ryan, 1959; Edwards, 1963; Rodger, 1965; Rodger 1967). When more than two treatment conditions are compared, then a type I error rate of $\alpha = .05$ can be set for each contrast, if they are orthogonal to one another and predicted before the data are collected. Each contrast is rejected by $F \geqslant F_{(1-\alpha)}$ from the traditional tables with $\nu_1 = 1$. These tests are exactly equivalent to t tests and α is the type I error rate for each decision made (Rodger, 1967, p.58). If, however, it is not possible to predict orthogonal contrasts, or if it is desired to look for differences after the data have been collected, then these tests are not statistically independent.

Rodger (1965, 1967) has developed a technique, the R technique, for testing any components suggested by scrutiny of the data with known error rates α. This is based on tables of $F_{(1-\alpha)}^{\nu_1}$ (Rodger, 1965) instead of the traditional $F_{(1-\alpha)}$. Thus in this complex design, the overall components are tested with $F_{(1-\alpha)}^{\nu_1}$ and if this is significant, then a search is made of the data for some significant contrasts, with the restriction of the number being made being no more than a-1. Because of the discrepancies (Chapter 1)

in the literature, it was not possible to make specific predictions about the contrasts before the experiment was commenced. A type I error rate of $\alpha = 0.05$ and a type II error rate of $\beta = 0.05$ were set. With an n = 5, these values allow the detection of a "true difference" between population means as large or larger than 2.35σ (Guenther, 1965) for comparisons between any two conditions (σ = population standard deviation) with these error rates.

3.2.7 Results

3.2.7.1 Nature of Evoked Activity

The amplitude of the responses studied was the peak-to-peak deflection incorporating both primary and slow wave activity occurring 250 msec after the commencement of the auditory stimulus. No attempt was made to analyse the different components of the evoked response.

One of the features of the response is the marked variation in response amplitude. This variation appeared to be more marked in the MG (see Fig.3-2). The variation in the peak-to-peak amplitudes appeared to be mostly due to fluctuations in the late components of the evoked responses. Fig. 3-2 illustrates the variation at the different areas and shows the change in overall amplitude following 95 minutes of repetitive stimulation. Similar results were observed in all placements.

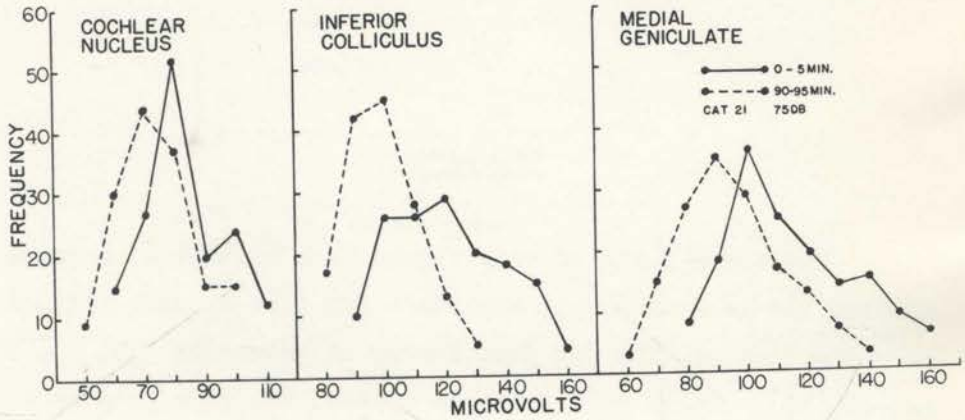
3.2.7.2 Cochlear Nucleus

When the microvolt and the percentage of control amplitudes data were plotted as a function of time with intensity levels as parameters (Fig.3-3), it was found that a small but consistent decrement occurred at each level of stimulus intensity. The greatest fall in the size of the amplitude appeared to occur in the first five minutes. In the curves for 75, 85 and 105 db stimuli, very little change occurred after the first period. In the 105 db curve, some variation was observed at the 40-45, 60-65, 80-85 minute recording periods. These divergences appeared to be due to variations in two placements. The reason for these changes is not known, since there seemed to be no correlated change in position or arousal state.

Figure 3-2

The top half of this figure shows a frequency plot of amplitudes recorded at the CN, IC and MG for the first and last five minutes of a recording session. These figures give some indication of the amplitude variation and the mean amplitude change after repetitive stimulation.

The lower half shows decrements in evoked potential amplitude over eight hours continuous stimulation by a 95 db, 20 msec click presented 1/2 sec.


COCHLEAR NUCLEUS
INFERIOR COLLICULUS
MEDIAL GENICULATE
0 - 5 MIN.
90-95 MIN.
CAT 21 75DB
FREQUENCY
MICROVOLTS

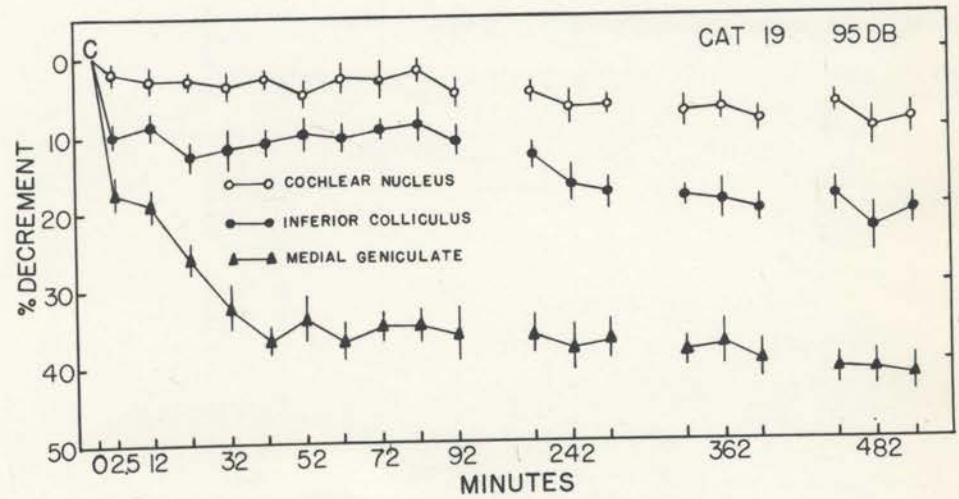
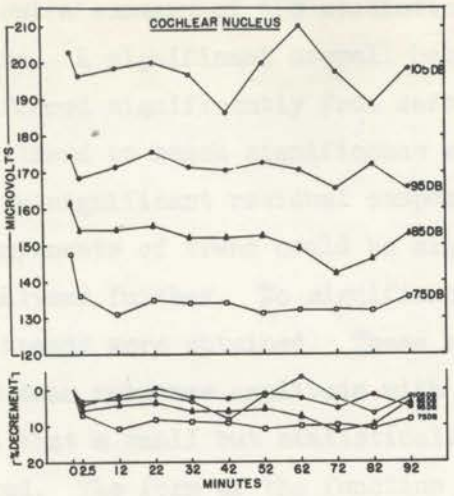
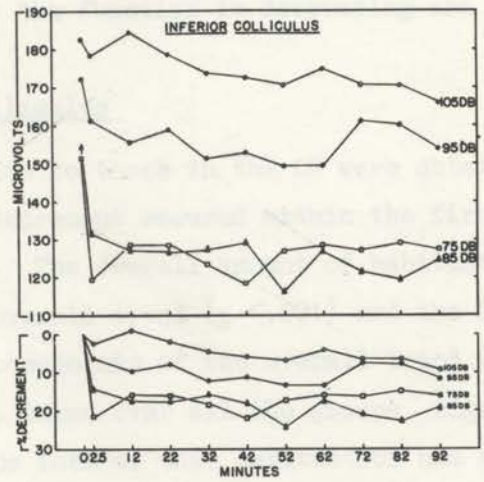

C
CAT 19 95 DB
COCHLEAR NUCLEUS
INFERIOR COLLICULUS
MEDIAL GENICULATE
% DECREMENT
MINUTES

Figure 3-3

The three parts of this figure each consist of a graph showing the mean microvolt amplitude for five animals plotted as a function of time for four levels of intensity (75, 85, 95 and 105 db). Below this graph is plotted the same data as a percentage of control amplitude. The top two graphs show the results for the CN, the middle two graphs show the results for the IC, and the bottom graphs show the results for the MG. Stimuli were presented at 1/2 sec.


COCHLEAR NUCLEUS
MICROVOLTS
%DECREMENT
MINUTES
0 2.5 12 22 32 42 52 62 72 82 92
105DB
95DB
85DB
75DB


INFERIOR COLLICULUS
MICROVOLTS
%DECREMENT
MINUTES
0 2.5 12 22 32 42 52 62 72 82 92
105DB
95DB
75DB
85DB

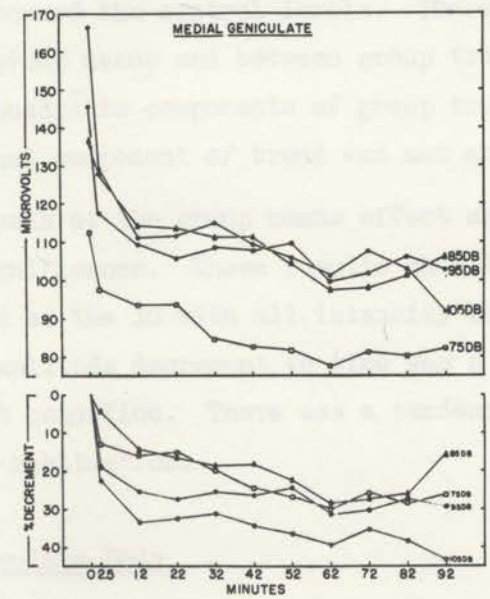

MEDIAL GENICULATE
MICROVOLTS
%DECREMENT
MINUTES
0 2.5 12 22 32 42 52 62 72 82 92
85DB
95DB
105DB
75DB

Table 4 sets out a summary of the statistical analysis on the trends in the percentage data. A significant overall habituation has occurred, as the overall trend differed significantly from zero slope ($p<.001$). The only component of overall trend to reach significance was the quadratic component, ($p<.05$). There was a significant residual component of trend which indicates that higher order components of trend could be significant. This residual component was not analysed further. No significant differences between treatment means and trends were obtained. These results indicate that the function relating CN mean response amplitude with time is similar at all intensity levels and that a small but statistically significant habituation decrement had occurred. The form of the function resembles a negative exponential, in that the function is decreasing and negatively accelerated.

3.2.7.3 Inferior Colliculus

Similar results to those in the CN were obtained in the IC. Once again the greatest decrement occured within the first five minutes of recording (Fig.3-3). The overall amount of habituation tended to be greater in this area. The overall trend ($p<.001$) and the linear ($p<.01$) and quadratic ($p<.05$) components of the overall trend were significant (Table 5). This indicates that, taken over all the groups, significant habituation has occurred and that the form of this habituation has a downward trend plus a tendency to return toward the control levels. There is a significant difference between group means and between group trends ($p<.05$), but neither the linear nor the quadratic components of group trends reaches significance; a significant residual component of trend was not analysed further.

Further analysis of the group means effect showed that no orthogonal contrast reached significance. These results show that significant overall habituation occurred at the IC with all intensity levels. The form of the function relating amplitude decrement to time was a negative exponential and was similar for each condition. There was a tendency for the least intense groups to show most habituation.

3.2.7.4 Medial Geniculate Body

The size of the decrement observed at this nucleus was much greater

TABLE 4

SUMMARY OF ORTHOGONAL TREND ANALYSIS

Cochlear Nucleus - Experiment 2

SOURCE	DF	SS	MS	F
OVERALL TREND	10	.089	.009	5.29 ***
Linear	(1)	.032	.032	5.92
Quadratic	(1)	.008	.008	8.17 *
Residual	(8)	.049	.006	5.13 ***
BETWEEN TREATMENT MEANS	3	.047	.136	1.99
BETWEEN TREATMENT TRENDS	30	.096	.002	0.68
BETWEEN SUBJECT MEANS	4	.113	.028	
SUBJECT BY TREATMENT MEANS	12	.816	.068	
BETWEEN SUBJECT TRENDS	40	.068	.002	
Linear	(4)	.022	.005	
Quadratic	(4)	.004	.001	
Residual	(32)	.042	.001	
SUBJECT BY TREATMENT TRENDS	120	.361	.003	
TOTAL	219	1.930		

*** significant at .001 level

** significant at .01 level

* significant at .05 level

Significant F values in this table were obtained from the table for the Protected Variance Ratio, $F_{(1-\alpha)\nu_1}$ in Rodger (1965).

TABLE 5

SUMMARY OF ORTHOGONAL TREND ANALYSIS

Inferior Colliculus - Experiment 2

SOURCE	DF	SS	MS	F
OVERALL TREND	10	.335	.034	5.21 ***
Linear	(1)	.128	.128	24.90 **
Quadratic	(1)	.065	.065	8.72 *
Residual	(8)	.142	.018	3.00 ***
BETWEEN TREATMENT MEANS	3	.811	.270	2.27 *
BETWEEN TREATMENT TRENDS	30	.157	.005	1.28 *
Linear	(3)	.010	.004	.29
Quadratic	(3)	.029	.010	1.90
Residual	(24)	.118	.005	1.67 **
BETWEEN SUBJECT MEANS	4	1.428	.355	
SUBJECT BY TREATMENT MEANS	12	1.428	.119	
BETWEEN SUBJECT TRENDS	40	.258	.006	
Linear	(4)	.020	.005	
Quadratic	(4)	.030	.007	
Residual	(32)	.208	.006	
SUBJECT BY TREATMENT TRENDS	120	.491	.004	
Linear	(12)	.141	.012	
Quadratic	(12)	.060	.005	
Residual	(96)	.290	.003	
TOTAL	219	4.901		

MEANS - ORTHOGONAL CONTRASTS

Largest Difference-75 db vs 105 db: $F = 1.69$ (df 1, 12)

75, 85 and 95 db vs 105 db: $F = 1.07$ (df 3, 12)

*** significant at .001 level

** significant at .01 level

* significant at .05 level

Significant F values in this table were obtained from the table for the Protected Variance Ratio, $F_{(1-\alpha)}$ in Rodger (1965).

than that observed at the IC or the CN (Fig.3-3). The general pattern was similar, with a rapid decrement early, but with a tendency for the means to continue decreasing over time.

Significant habituation occurred since the overall trend differed significantly from zero slope ($p<.001$) and the linear and quadratic components ($p<.001$) also differed from zero slope (Table 6). A significant difference between treatment means ($p<.05$) and between treatment trends ($p<.05$) occurred. A further analysis of the mean effect showed that the combined 75, 85 and 95 db groups differed from the 105 db group ($p<.05$). A significant linear component of group trends ($p<.05$) was obtained, but only the contrast of the combined 75, 85 and 95 db versus the 105 db group was significant ($p<.05$). Significant residuals of trend were obtained but these were not analysed further.

The results show that significant overall habituation occurred at the MG to all intensity levels. The form of the function relating the amplitude decrement to time was similar for each condition and was a negative exponential. There was a tendency for the most intense stimulus to produce the largest decrement.

3.2.7.5 Control Study

To control for the possibility that much larger decrements would occur with much longer periods of stimulation, two cats were tested for eight hours (14,000 consecutive stimuli) with a 95 db stimulus at a rate of 1/2 sec. For both cats the changes in mean response amplitude for the first 95 min. were similar to those reported above (Fig.3-2). For the next $6\frac{1}{2}$ hours, one animal showed a further gradual decline in amplitude at the MG, the IC and the CN (Fig.3-2). With the other animal, the MG response continued to decline, but no change was observed in the IC and there was a return to control levels at the CN. These results indicate that reductions of response amplitude to noise level are unlikely to occur.

TABLE 6

SUMMARY OF ORTHOGONAL TREND ANALYSIS

Medial Geniculate - Experiment 2

SOURCE	DF	SS	MS	F
OVERALL TREND	10	1.805	.181	44.63 ***
Linear	(1)	1.141	1.141	61.73 ***
Quadratic	(1)	.368	.368	132.82 ***
Residual	(8)	.295	.037	18.50 ***
BETWEEN TREATMENT MEANS	3	.660	.220	4.99 **
BETWEEN TREATMENT TRENDS	30	.183	.006	1.74
Linear	(3)	.024	.008	3.67 *
Quadratic	(3)	.021	.007	.48
Residual	(26)	.140	.006	2.00 ***
BETWEEN SUBJECT MEANS	4	.359	.090	
SUBJECT BY TREATMENT MEANS	12	.528	.044	
BETWEEN SUBJECT TRENDS	40	.162	.004	
Linear	(4)	.074	.018	
Quadratic	(4)	.011	.003	
Residual	(32)	.077	.002	
SUBJECT BY TREATMENT MEANS	120	.420	.004	
Linear	(12)	.086	.007	
Quadratic	(12)	.062	.005	
Residual	(96)	.292	.003	
TOTAL	219	4.118		

MEANS - ORTHOGONAL CONTRASTS

Largest Difference 75 db vs 105 db: $F = 1.60$ (df 1, 12)

75, 85 and 95 db vs 105 db: $F = 3.40$ (df 3, 12)*

LINEAR - ORTHOGONAL CONTRASTS

Largest Difference 85 db vs 105 db: $F = 1.13$ (df 1, 12)

75, 85 and 95 db vs 105 db: $F = 19.20$ (df 3, 12)***

*** significant at .001 level

** significant at .01 level

* significant at .05 level

Significant F values in this table were obtained from the table for Protected Variance Ratio, $F_{(1-\alpha)\nu_1}$ in Rodger (1965).

3.2.8 Histology

All electrode placements were confirmed using techniques already described. Figure 3-4 shows the approximate location of each electrode.

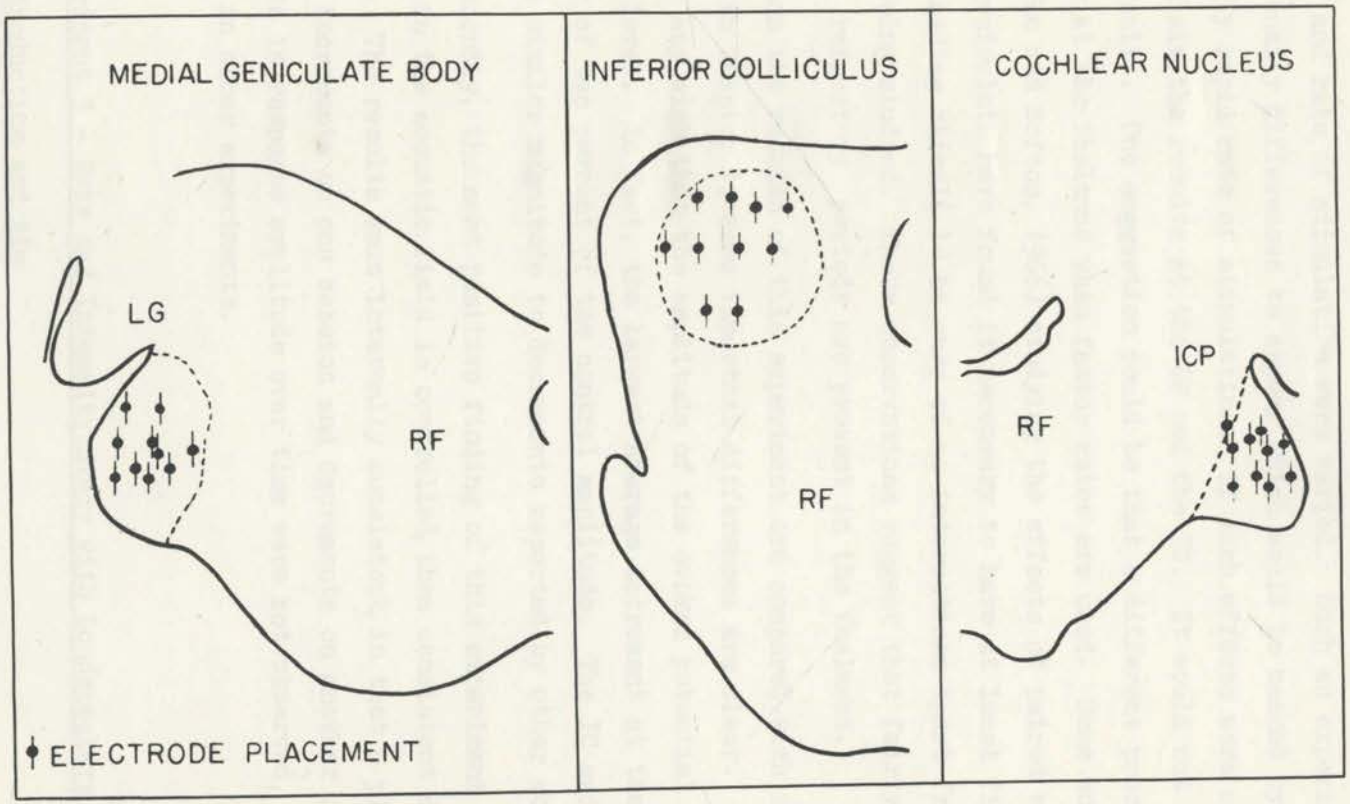
3.2.9 Discussion

The results show that consistent auditory evoked potential decrements can be obtained at the CN, IC and MG in response to auditory stimulation at 1/2 sec. The size of the decrement was greatest at the MG and least at the CN. The IC gave intermediate results. In each case, the form of the group function resembled a negative exponential which is one of the characteristics of BRH, although not a critical characteristic. The effects of stimulus intensity are equivocal. All placements showed decrements at all intensity levels. There was a tendency at the IC for the least intense stimulus to produce a greater decrement, but none of the individual group comparisons yielded a significant result. In the MG, the most intense stimulus produced a greater decrement than the combined less intense stimuli. This is in the opposite direction to any expectation based on BRH. The CN showed no difference due to stimulus intensity. This study does not present any clear-cut evidence that stimulus intensity influences AEPH in the same way as it influences BRH. One possible explanation is that only the IC is a site for habituation and that the CN is not an area influenced by intensity in this way. The intensity results at the MG are in the opposite direction to typical BRH effects and this might suggest that habituation to auditory stimuli is a pre-thalamic phenomenon. But it is felt that while these possibilities should be kept open, there could be other explanations of these results.

It is possible, however, that the failure to find intensity effects comparable to those found with behavioural response habituation is due to the rate of stimulation employed. In this experiment, a fairly rapid rate of 1/2 sec. was used and there is some evidence of faster habituation to faster rates of presentation of auditory stimuli (Prosser and Hunter, 1936). Thus it is possible that fairly rapid habituation occurs to all the intensity levels employed in this

Figure 3-4

The approximate location of electrodes in the medial geniculate body, inferior colliculus and the cochlear nucleus in the animals used in Experiment 2.


MEDIAL GENICULATE BODY
INFERIOR COLLICULUS
COCHLEAR NUCLEUS
LG
RF
RF
RF
ICP
ELECTRODE PLACEMENT

experiment when rates of stimulation reach a rapid level. If this were the case, then this experiment would not be a critical test of the influence of stimulus intensity. What is required is an experiment in which both stimulus intensity and rate of stimulation were varied. Such an experiment might allow intensity differences to appear which would be masked by the effects of a fairly rapid rate of stimulation. If such effects were obtained it would explain the results at the CN and the IC. It would not explain away the MG results. One suggestion could be that a different process is brought into play at the thalamus when faster rates are used. Some workers (Bishop, 1964; Burke and Sefton, 1966) studying the effects of paired stimuli on the lateral geniculate have found it necessary to have at least five seconds between leading stimuli to be sure of no interactions apart from the paired stimuli being studied. These observations suggest that fairly long relative refractory periods are present in the thalamus.

When the results of this experiment are compared with the studies examined in Chapter 1, some important differences are clear. Firstly, there was never any sign that the amplitude of the evoked potential would decrease to noise level. In fact, the largest average decrement at the CN was of the order of ten percent of the control amplitude. The IC and MG decrements were of a similar magnitude to decrements reported by other workers.

Secondly, the most positive finding of this experiment is that when movement in the acoustic field is controlled, then consistent decrements are obtained. The results were internally consistent, in that a placement did not show increments on one session and decrements on another or vice versa. Increments in response amplitude over time were not observed, as have been reported in other experiments.

3.3 Experiment 3 - Rate and Intensity Study with Loudspeakers

3.3.1 Introduction and Aim

In the discussion of Experiment 2 it was suggested that the relatively rapid (1/2 sec) rate of stimulation might have hidden an intensity effect by producing marked AEPH at each intensity level. While this is a possible explanation for the effects at the CN and the IC, it would not explain the

differences between BRH and the results obtained at the MG. It might be possible, however, that two processes are operating at the level of the thalamus: a refractory process and an habituation process. Thus one way of attempting to disentangle these factors would be to manipulate both rate of stimulation and stimulus intensity within the one experiment.

The aim of this experiment was to vary systematically stimulus intensity and rate of stimulus presentation, and to determine the relationship between these variables and evoked response amplitude changes recorded in the CN, IC and the MG during repetitive auditory stimulation.

3.3.2 Subjects and Electrode Implantation

Using techniques described in 2.2.4 and 3.2.2, bipolar electrodes were implanted in the MG, IC and CN of seventeen cats. The same criteria of amplitude and latency were employed as in section 3.2.2, and from this population of placements, five placements were selected in the IC, six in the CN and seven in the MG for the experiment.

3.3.3 Stimulus Parameters

The auditory stimulus was once again a 20 msec "click" or pulse burst generated and measured as described in 3.2.3. Peak sound-pressure levels of 85 and 105 db re .0002 microbar (section 3.2.3) were chosen. Five rates of stimulation were employed: 10/1 sec, 1/1 sec, 1/5 sec, 1/10 sec and 1/20 sec.

The decision to employ 85 and 105 db as intensities was based on Experiment 2. There did not appear to be marked differences between the magnitude of decrement with 75 and with 85 db stimuli. It was easier to obtain a signal-to-noise ratio of 1/2 with 85 db and this still provided a good range of intensity when compared with 105 db. The five rates of stimulation were chosen in light of the considerations raised in section 3.3.1.

3.3.4 Test Environment, Design and Data Collection

The same test cage (see section 2.4.2) was employed in this experiment. Once again, each animal was placed in the cage for periods of one to two

hours to accustom it to the experimental situation. Similar recording techniques were used (3.2.4). Each animal was tested under each condition of intensity and rate or under ten test conditions. The order of testing was randomised over the cats. From the results of the previous experiment, it was decided to reduce the period of the experimental session from 95 to 20 minutes. It was felt that this would make it easier to obtain complete records that were acceptable by the spatial criteria set out earlier. That is, the animal should remain in the one quadrant throughout the session and there should be little change in head position in the vertical dimension. At least seven days were allowed to elapse between consecutive sessions for any one cat. All the responses were recorded for each session.

In this experiment a new type of control level was introduced. When the animal was placed in the box and allowed to settle, then six stimuli were given over a period of ten minutes. The interval between the stimuli was random but with the restraint that no interval should be smaller than 30 seconds. It was felt that this gave a more valid pre-habituation control.

3.3.5 Data Reduction

Once again the peak-to-peak deflection of the evoked responses was measured with a ruler to the nearest mm and then transformed to microvolts. The first minute of recording was measured and every alternate one minute period. The mean response amplitude was calculated for each one minute period. This mean figure was then expressed as a percentage of the control microvolt amplitude. Any changes in the records due to movement or vocalisation were dropped from the analysis.

3.3.6 Statistical Predictions and Analysis

This experiment involved five rates of stimulation at two levels of intensity. This gives a total of ten stimulus conditions. Consequently, there are nine degrees of freedom between groups. It was predicted from the results of Experiment 2 that there would be an intensity effect at the IC, and at the MG, independently of any rate

effect. Furthermore, it was predicted that there would be no intensity effect at the CN. Lastly, it was predicted on the basis of the discussion in 3.2.9 that a rate effect would be present in each nucleus. The possibility that there would be greater effects with less intense stimuli at more rapid rates would be tested by the interaction term.

The modified test of trend allows a test of all these predictions for means and linear and quadratic trend components with the following degrees of freedom: Intensity (1), Rate (4),and Interaction of Rate and Intensity (4). This exhausts the degrees of freedom available. In addition, it will be predicted that if the data from the two conditions of slowest rate (85 db, 1/20 sec; 105 db, 1/20 sec) are combined this will not differ from zero slope when tested for linear trend and quadratic trend, that is, that there will be no decrement produced by stimuli occurring at this rate.

These data were analysed using the modified Grant's (1956) test of trend. Similar steps were taken to those specified in section 3.2.6. Once again it was clear that the test was robust to lack of homogeneity of variance and only the percentage data were used for further analysis. Type I and Type II error rates of 0.05 were set.

3.3.7 Histological Verification

All placements were verified using techniques described in section 2.4.5. Figures 3-6 and 3-7 show the approximate location of the electrodes in the CN, IC and MG.

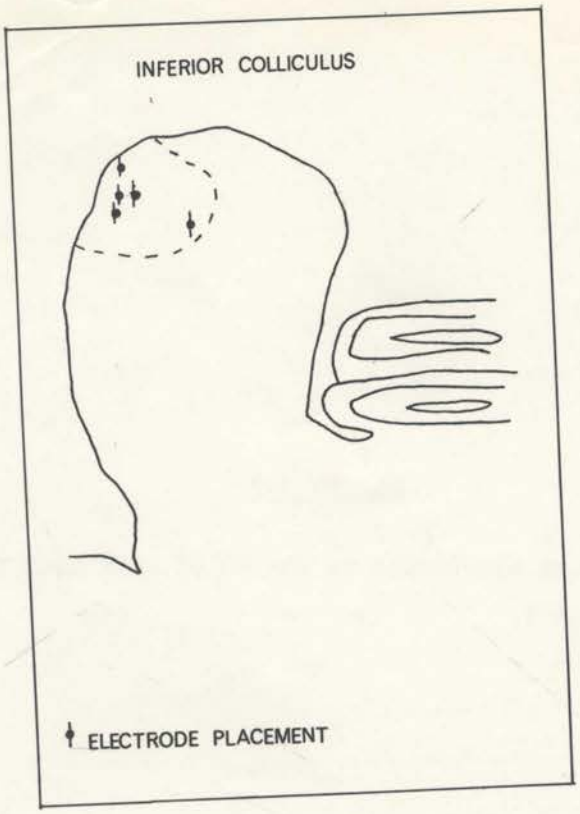
3.3.8 Results

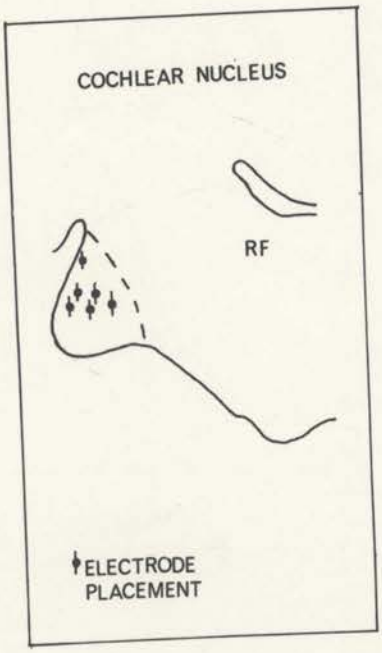
3.3.8.1 Nature of Evoked Activity

The peak-to-peak amplitude studied was the deflection occurring 250 msec after the stimulus. It included both primary and slow wave activity. No attempt was made to analyse the different components of the evoked response.

Figure 3-6

Location of electrodes in the CN and IC of cats used in Experiment 3.


INFERIOR COLLICULUS
ELECTRODE PLACEMENT


COCHLEAR NUCLEUS
RF
ELECTRODE PLACEMENT

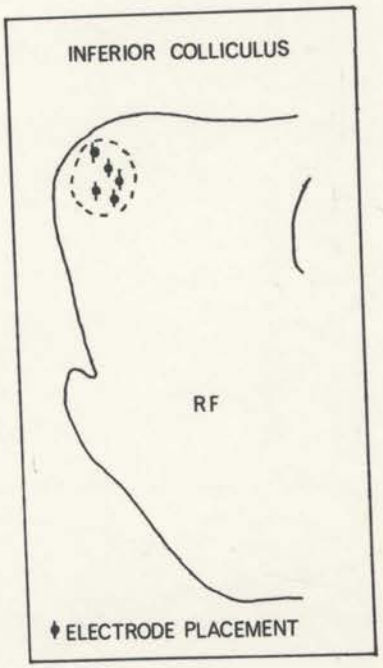
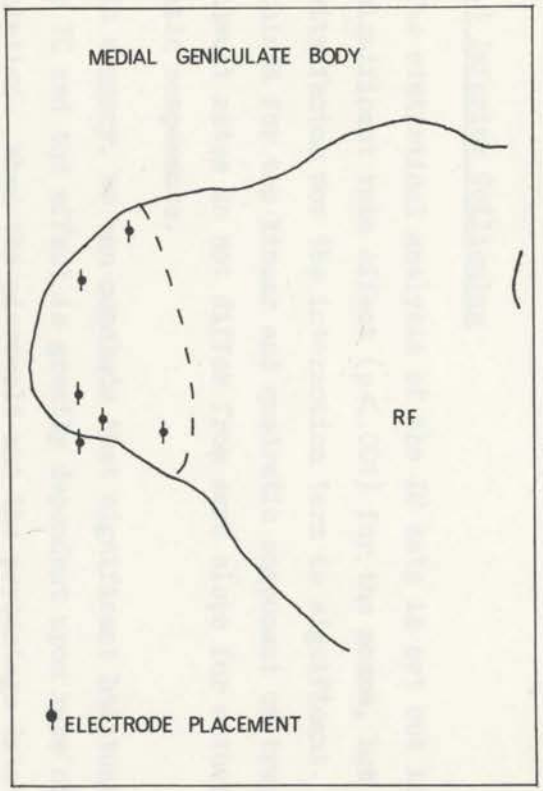
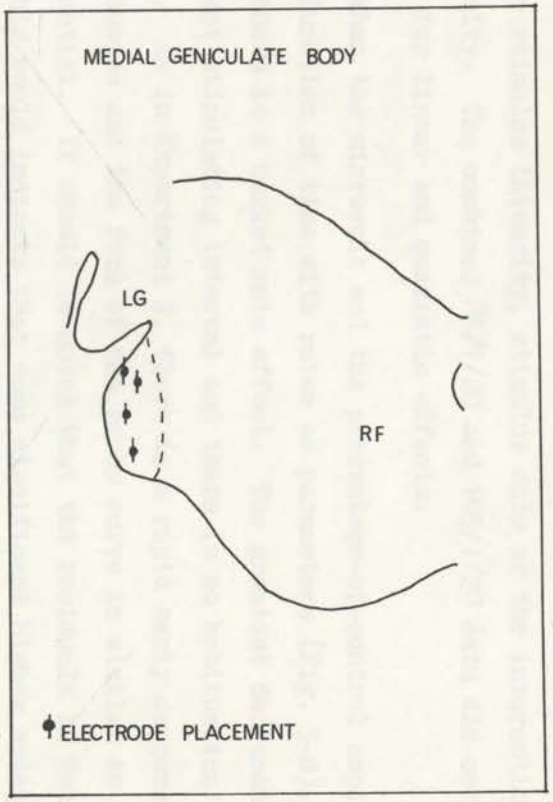

INFERIOR COLLICULUS
RF
ELECTRODE PLACEMENT

Figure 3-7

Location of electrodes in the MG of cats used in Experiment 3.


MEDIAL GENICULATE BODY
RF
ELECTRODE PLACEMENT


MEDIAL GENICULATE BODY
LG
RF
ELECTRODE PLACEMENT

3.3.8.2 Cochlear Nucleus

The results of the statistical analysis are set out in Table 7. There is a significant rate effect for means ($p<.001$), but no difference due to intensity. The interaction term was also not significant. With both the linear and quadratic component of trends, there were no significant effects due to stimulus intensity, stimulus rate or the interaction of rate and intensity. The combined 85/1/20 and 105/1/20 data did not differ from zero slope for linear and quadratic effects.

When the microvolt and the percentage-of-control amplitudes are plotted as a function of time with rates as parameters (Fig. 3-8), it can be seen that there is a marked rate effect. The greatest decrement occurs with the shortest stimulating interval and there is no habituation with the slowest rates. As in Experiment 2, there is a rapid early decrement in the first few minutes and the form of the group curve is similar to a negative exponential. It should be noted that the residuals in Table 7 are significant and this would indicate that some significant higher order components of trend are present.

3.3.8.3 Inferior Colliculus

The statistical analysis of the IC data is set out in Table 8. There is a significant rate effect ($p<.001$) for the means, but neither the intensity factor nor the interaction term is significant. A similar result is obtained for the linear and quadratic component of trend. The combined two slowest rates do not differ from zero slope for either the linear or quadratic components.

In summary, we can conclude that significant habituation has occurred in the IC and the effect is greatly dependent upon rate of stimulus presentation. When the microvolt and the percentage data are plotted as a function of time with rates as parameters (Fig. 3-9), the rate effect is quite apparent, with habituation absent at the slowest rate of stimulation. Once again there is a rapid early decrement and the form of the group curves is similar to a negative exponential. The residuals are significant (Table 8), and this indicates that higher order components of trend are probably significant. No attempt was made to analyse these.

TABLE 7

SUMMARY OF ORTHOGONAL TREND ANALYSIS

Cochlear Nucleus - Experiment 3

SOURCE	DF	SS	MS	F
OVERALL TREND	10	.771	.077	14.49 ***
Linear	(1)	.237	.237	22.20 **
Quadratic	(1)	.156	.156	8.70 *
Residual	(8)	.378	.047	15.60 ***
BETWEEN TREATMENT MEANS	9	4.110	.457	4.63 ***
Rate	(4)	3.838	.959	9.73
Intensity	(1)	.025	.025	.25
Rate x Intensity	(4)	.248	.062	.63
BETWEEN TREATMENT TRENDS	90	.663	.007	1.60 **
Linear	9	.102	.011	..93
Rate	(4)	.046	.011	.94
Intensity	(1)	.029	.029	2.37
Rate x Intensity	(4)	.028	.007	.56
Quadratic	9	.122	.014	1.44
Rate	(4)	.085	.021	2.24
Intensity	(1)	.002	.002	.25
Rate x Intensity	(4)	.035	.008	.92
Residual	(72)	.439	.006	2.00 ***
BETWEEN SUBJECT MEANS	5	.493	.098	
SUBJECT BY TREATMENT MEANS	45	4.435	.098	
BETWEEN SUBJECT TRENDS	50	2.659	.005	
Linear	(5)	.053	.011	
Quadratic	(5)	.090	.018	
Residual	(40)	.123	.003	
SUBJECT BY TREATMENT TRENDS	450	2.075	.005	
Linear	(45)	.550	.012	
Quadratic	(45)	.425	.009	
Residual	(360)	1.100	.003	
TOTAL	659	12.813		

Linear - 85/1/20 + 105/1/20 vs zero slope: F = .06 (1,45)

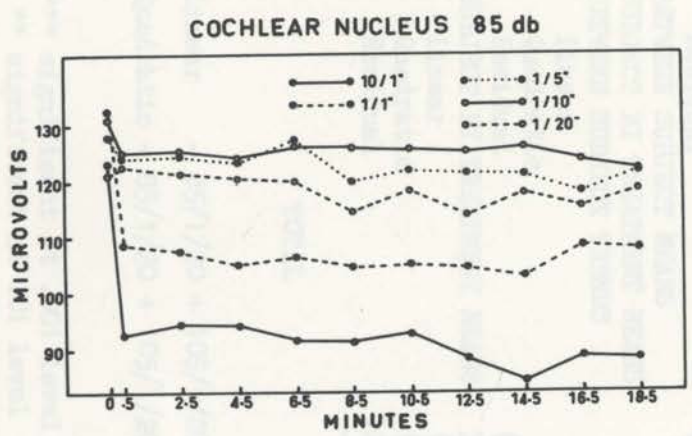
Quadratic - 85/1/20 + 105/1/20 vs zero slope: F = .33 (1,45)

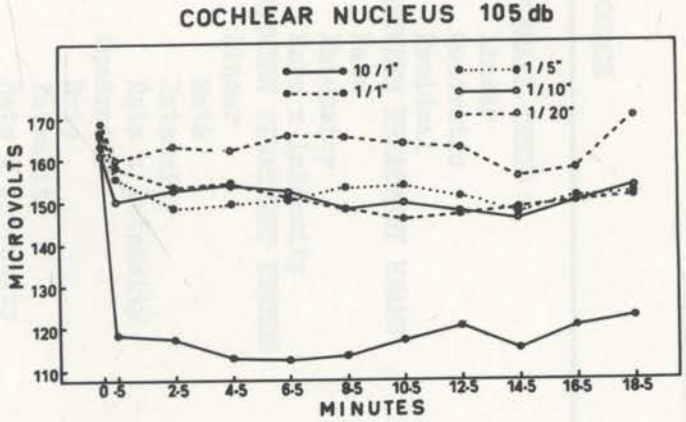
*** significant at .001 level
** significant at .01 level
* significant at .05 level

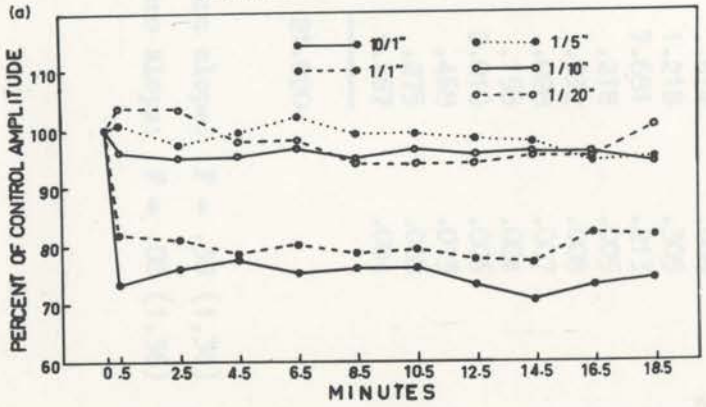
Significant F values obtained from Variance Ratio table, $F(1-\alpha)$, in Rodger (1965)

Figure 3-8

Graphs showing CN microvolt amplitude and percentage of control data plotted as a function of time. The first column contains plots for 85 db clicks and the second column contains plots for 105 db clicks.


COCHLEAR NUCLEUS 85 db
10/1"
1/5"
1/1"
1/10"
1/20"
MICROVOLTS
130
120
110
100
90
0·5
2·5
4·5
6·5
8·5
10·5
12·5
14·5
16·5
18·5
MINUTES


COCHLEAR NUCLEUS 105 db
10/1"
1/5"
1/1"
1/10"
1/20"
MICROVOLTS
170
160
150
140
130
120
110
0·5
2·5
4·5
6·5
8·5
10·5
12·5
14·5
16·5
18·5
MINUTES


(a)
COCHLEAR NUCLEUS 85 db
10/1"
1/5"
1/1"
1/10"
1/20"
PERCENT OF CONTROL AMPLITUDE
110
100
90
80
70
60
0·5
2·5
4·5
6·5
8·5
10·5
12·5
14·5
16·5
18·5
MINUTES

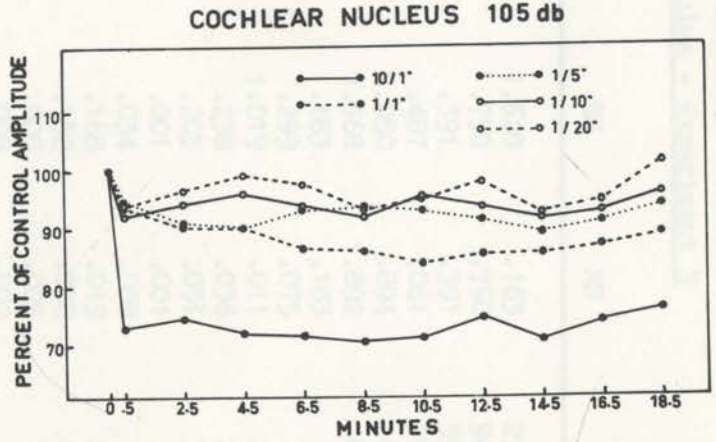

COCHLEAR NUCLEUS 105 db
10/1"
1/5"
1/1"
1/10"
1/20"
PERCENT OF CONTROL AMPLITUDE
110
100
90
80
70
0·5
2·5
4·5
6·5
8·5
10·5
12·5
14·5
16·5
18·5
MINUTES

TABLE 8

SUMMARY OF ORTHOGONAL TREND ANALYSIS

Inferior Colliculus - Experiment 3

SOURCE	DF	SS	MS	F
OVERALL TREND	10	1.628	.163	29.92 ***
Linear	(1)	.750	.750	94.03 ***
Quadratic	(1)	.391	.391	55.30 ***
Residual	(8)	.487	.061	15.00 ***
BETWEEN TREATMENT MEANS	9	8.050	.894	4.19 **
Rate	(4)	7.568	1.892	8.87 ***
Intensity	(1)	.183	.183	.86
Rate x Intensity	(4)	.299	.075	.35
BETWEEN TREATMENT TRENDS	90	1.019	.011	2.03 ***
Linear	(9)	.238	.036	2.03
Rate	(4)	.203	.051	3.91 **
Intensity	(1)	.001	.001	.06
Rate x Intensity	(4)	.034	.008	.65
Quadratic	(9)	.168	.019	2.44 *
Rate	(4)	.152	.038	4.98 **
Intensity	(1)	.008	.008	1.05
Rate x Intensity	(4)	.008	.002	.25
Residual	(72)	.614	.008	2.00 **
BETWEEN SUBJECT MEANS	4	1.218	.305	
SUBJECT BY TREATMENT MEANS	36	7.681	.213	
BETWEEN SUBJECT TRENDS	40	.218	.005	
Linear	(4)	.032	.008	
Quadratic	(4)	.028	.007	
Residual	(32)	.158	.004	
SUBJECT BY TREATMENT MEANS	360	2.010	.006	
Linear	(36)	.468	.013	
Quadratic	(36)	.275	.008	
Residual	(288)	1.267	.004	
TOTAL	549	21.820		

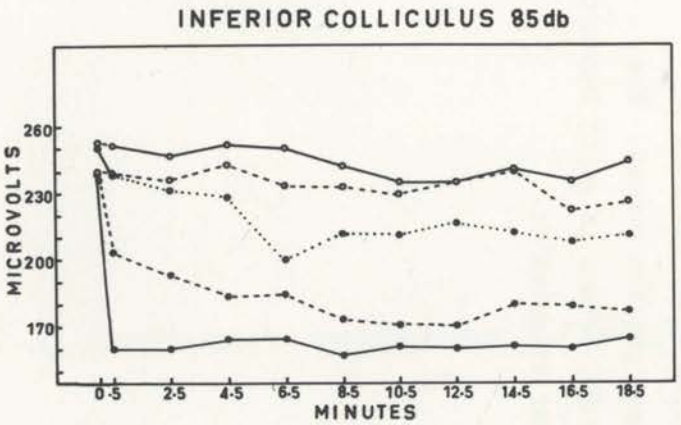
Linear - 85/1/20 + 105/1/20 vs zero slope: F = .38 (1,36)

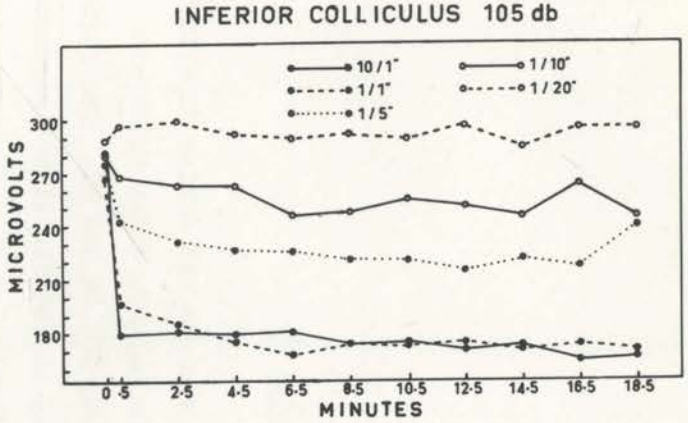
Quadratic - 85/1/20 + 105/1/20 vs zero slope: F = .08 (1,36)

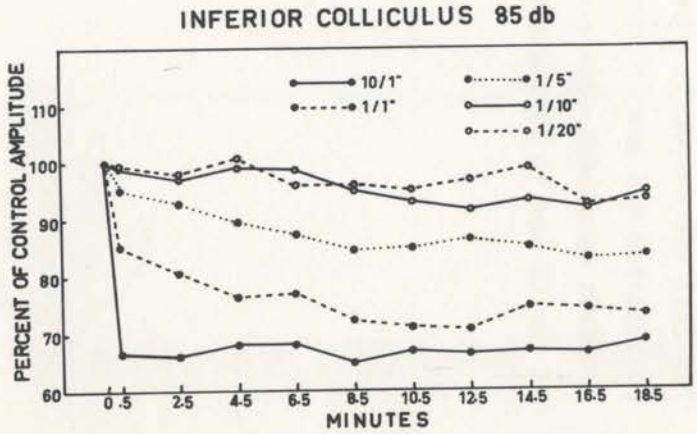
*** significant at .001 level
** significant at .01 level
* significant at .05 level

Figure 3-9

Microvolt and percentage amplitude data for the IC plotted as a function of time. The graphs on the left show data from 85 db clicks given at five rates of stimulation. The graphs on the right show plots of similar data obtained to 105 db clicks.


INFERIOR COLLICULUS 85db
MICROVOLTS
260
230
200
170
0·5
2·5
4·5
6·5
8·5
10·5
12·5
14·5
16·5
18·5
MINUTES


INFERIOR COLLICULUS 105 db
10/1"
1/10"
1/1"
1/20"
1/5"
MICROVOLTS
300
270
240
210
180
0·5
2·5
4·5
6·5
8·5
10·5
12·5
14·5
16·5
18·5
MINUTES


INFERIOR COLLICULUS 85 db
10/1"
1/5"
1/1"
1/10"
1/20"
PERCENT OF CONTROL AMPLITUDE
110
100
90
80
70
60
0·5
2·5
4·5
6·5
8·5
10·5
12·5
14·5
16·5
18·5
MINUTES

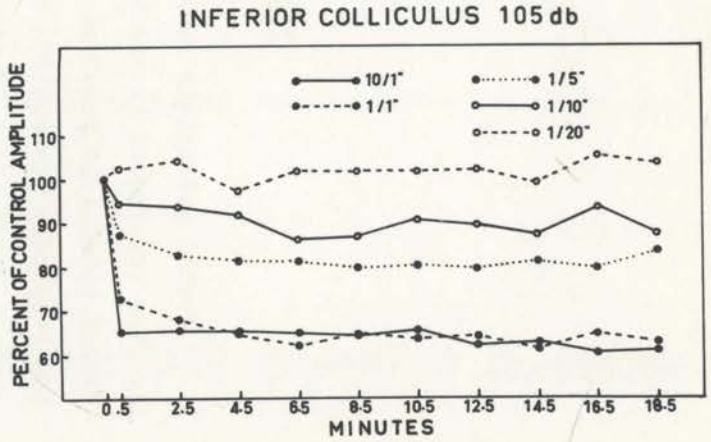
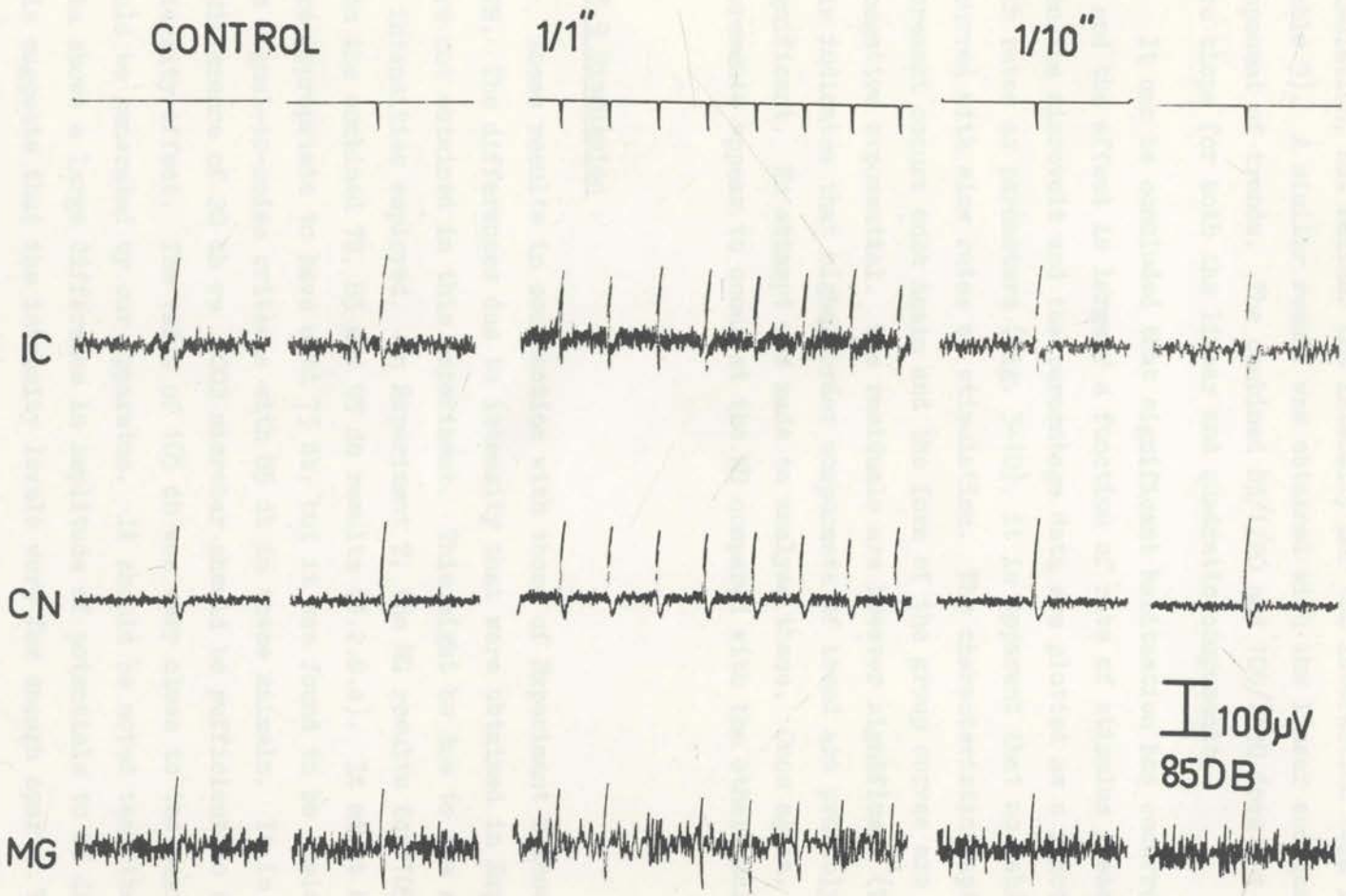

INFERIOR COLLICULUS 105 db
10/1"
1/5"
1/1"
1/10"
1/20"
PERCENT OF CONTROL AMPLITUDE
110
100
90
80
70
60
0·5
2·5
4·5
6·5
8·5
10·5
12·5
14·5
16·5
18·5
MINUTES

Figure 3-10A

Evoked potentials recorded from one cat during Experiment 3. The top record is the stimulus marker, under which are evoked potentials recorded from the IC, CN and MG. The first two responses are control stimuli, the middle eight responses were recorded after 20 minutes stimulation at one per second. The last two responses were recorded after the rate of stimulation had been changed to one per ten second. The greatest decrement occurs at the MG. There is rapid recovery at the CN and IC during the slower stimulation.


CONTROL
1/1"
1/10"
IC
CN
MG
100μV
85DB

3.3.8.4 Medial Geniculate Body

There is a significant effect ($p<.001$) in the MG means due to rate of stimulation, but neither the intensity nor the interaction term is significant (Table 9). A similar result was obtained with the linear and quadratic component of trends. The combined 85/1/20 and 105/1/20 does not differ from zero slope for both the linear and quadratic components.

It can be concluded that significant habituation has occurred in the MG and the effect is largely a function of rate of stimulus presentation. When the microvolt and the percentage data are plotted as a function of time, with rates as parameters (Fig. 3-10), it is apparent that no habituation has occurred with slow rates of stimulation. The characteristic rapid early decrement occurs once again and the form of the group curves are similar to a negative exponential. The residuals are however significant (Table 9) and this indicates that higher order components of trend are probably also significant. No attempt was made to analyse these. Once again, larger decrements appear to occur at the MG compared with the other nuclei.

3.3.9 Discussion

These results in conjunction with those of Experiment 2 show consistent AEPH. The differences due to intensity that were obtained in Experiment 2 were not obtained in this experiment. This might be due to the smaller range of intensities employed. In Experiment 2, the MG results for 105 db differed from the combined 75, 85 and 95 db results (3.2.8.4). It might have been more appropriate to have used 75 db, but it was found to be easier to reach the signal-to-noise criteria with 85 db in these animals. It is felt that a difference of 20 db re .0002 microbar should be sufficient to show up an intensity effect. The value of 105 db was very close to the maximum that could be generated by our apparatus. It should be noted that the microvolt data shows a large difference in amplitude of potentials to 85 db and 105 db. This suggests that the intensity levels were far enough apart. The prediction that there would be no intensity effect at the CN was supported.

There is clearly a strong rate effect in these results at each placement. In general, there was no decrement with the slowest rates of stimulation. It

TABLE 9

SUMMARY OF ORTHOGONAL TREND ANALYSIS

Medial Geniculate - Experiment 3

SOURCE	DF	SS	MS	F
OVERALL TREND	10	2.781	.278	35.87 ***
Linear	(1)	1.267	1.267	64.63 ***
Quadratic	(1)	.563	.563	64.38 ***
Residual	(8)	.951	.118	19.60 ***
BETWEEN TREATMENT MEANS	(9)	16.140	1.793	31.61 ***
Rate	(9)	15.966	3.992	70.35 ***
Intensity	(1)	.115	.115	2.03
Rate x Intensity	(4)	.058	.015	.26
BETWEEN TREATMENT TRENDS	90	2.068	.023	3.73 ***
Linear	9	.182	.020	1.74
Rate	(4)	.146	.037	3.15 *
Intensity	(1)	.002	.002	.19
Rate x Intensity	(4)	.034	.008	.72
Quadratic	(9)	.430	.048	6.18 ***
Rate	(4)	.376	.094	12.17 ***
Intensity	(1)	.009	.009	1.18
Rate x Intensity	(4)	.045	.011	1.45
Residual	(72)	1.458	.020	4.00 ***
BETWEEN SUBJECT MEANS	6	.761	.127	
SUBJECT BY TREATMENT MEANS	54	3.064	.057	
BETWEEN SUBJECT TRENDS	60	.469	.008	
Linear	(6)	.118	.020	
Quadratic	(6)	.052	.009	
Residual	(48)	.299	.006	
SUBJECT TREATMENT TRENDS	540	3.324	.006	
Linear	(54)	.626	.012	
Quadratic	(54)	.417	.008	
Residual	(432)	2.280	.005	
TOTAL	769	28.610		

Linear - 85/1/20 + 105/1/20 vs zero slope: F = 1.07 (1,54)

Quadratic - 85/1/20 + 105/1/20 vs zero slope: F = .02 (1,54)

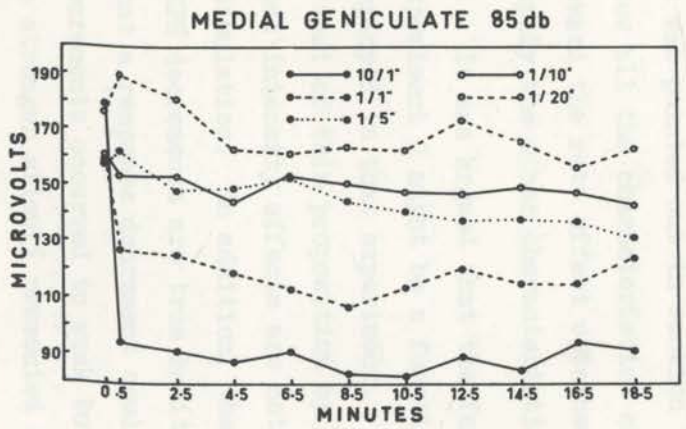
*** significant at .001 level
** significant at .01 level
* significant at .05 level

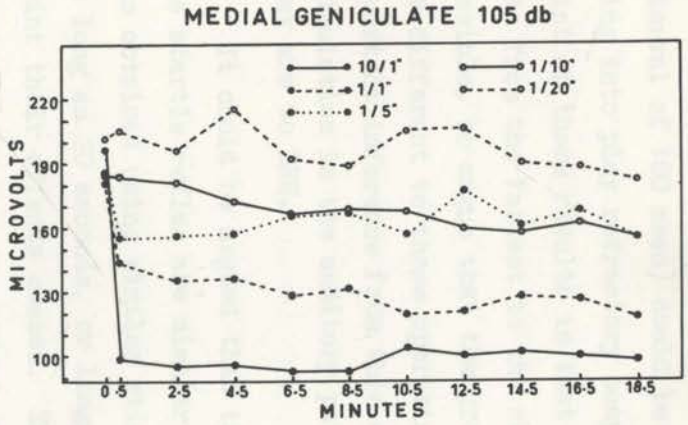
Figure 3-10

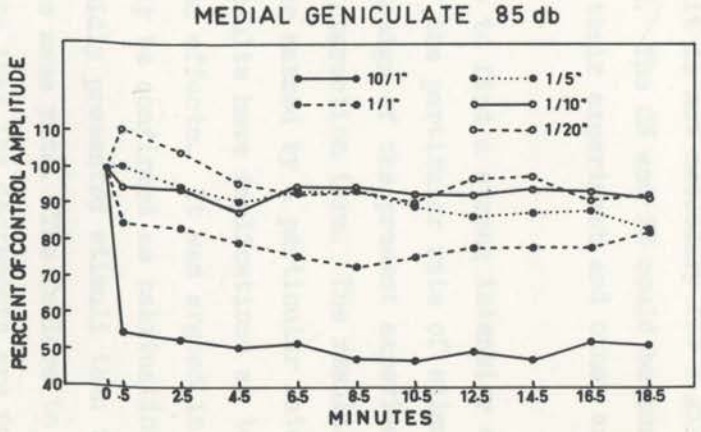
Microvolt and percentage amplitude data for the medial geniculate plotted as a function of time -

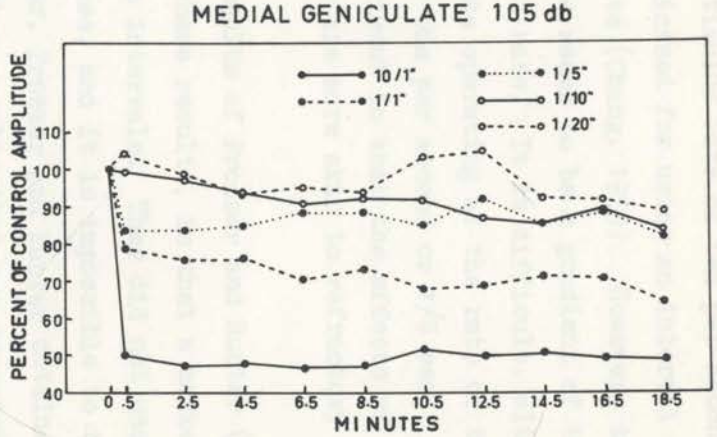
(a) the graphs in the first column show the data for 85 db clicks at five rates of stimulation;

(b) the graphs in the second column show plots of similar data obtained to 105 db clicks.


MEDIAL GENICULATE 85 db
MICROVOLTS
MINUTES
10/1"
1/1"
1/5"
1/10"
1/20"


MEDIAL GENICULATE 105 db
MICROVOLTS
MINUTES
10/1"
1/1"
1/5"
1/10"
1/20"


MEDIAL GENICULATE 85 db
PERCENT OF CONTROL AMPLITUDE
MINUTES
10/1"
1/1"
1/5"
1/10"
1/20"


MEDIAL GENICULATE 105 db
PERCENT OF CONTROL AMPLITUDE
MINUTES
10/1"
1/1"
1/5"
1/10"
1/20"

would seem that AEPH does not occur with interstimulus intervals in excess of 20 seconds. The choice of the stimulus rate of ten per second (or an interval of 100 msec) could be criticised for using an interval that might bring into play refractory components (Chang, 1959). However, the important point of these results is that there seems to be a gradient of the effect of rate from the fastest to the slowest rate. It is difficult, although possible, to argue that the processes operating at the rate of ten per second are different to those operating at one per second or 1/5 sec. Thus the important inference from this experiment is that the effects of repetitive stimulation in the auditory pathway are more akin to refractory effects than they are to BRH.

It could be argued that the results of Prosser and Hunter (1936) with the startle reflex are similar to these results, in that a marked rate effect was obtained using similar stimulus intervals. They did not use intervals as long as 20 seconds, or longer ones, and it is impossible to tell at what point their effects ceased. However, Prosser and Hunter obtained a significant intensity effect and other results that are expected for BRH. As was pointed out in section 1.2, it is not necessary for a given area to show all the characteristics of BRH. The CN and IC could be contributing toward the rate effect obtained in their experiment and other areas might supply the other characteristics.

It was argued that the failure to find a strong intensity effect in Experiment 2 might be a function of the particular rate of stimulation employed in that experiment. The design of the present experiment allowed a test of this proposition by the interaction term. The results indicate that intensity effects are not being masked by a particular rate of stimulation. In addition, these results have implications as to whether AEPH decrements are true habituation effects. It was argued in section 1.1.2 that a response decrement could only be confirmed as habituation if greater decrements occurred to weak but rapidly presented stimuli than those occurring to stronger stimuli presented at the same rate. The failure to find a significant interaction term suggests that the decrements are not due to habituation.

What sort of central processes could be producing an apparent refractory

effect for as long as five seconds at least? It is difficult to give a precise answer to this question, but there is evidence of central inhibitory processes which last longer than 100 msecs (Sefton and Burke, 1965). Thus one possible interpretation of the data from this experiment is that the observed decrements are due to intrinsic, long lasting inhibitory mechanisms, similar to those proposed by Andersen et al (1964) in the ventrobasal complex of the cat thalamus and Sefton and Burke (1965) in the lateral geniculate nucleus of the rat. Such mechanisms may account for a large part of the response decrements observed in the auditory pathway following repetitive stimulation. Thus we may speculate that each auditory stimulus evoked a field potential which terminates in a long lasting period (of the order of seconds) of depressed excitability and, as a consequence, another stimulus occurring during this time will evoke a field potential, the amplitude of which would be directly related to the time elapsed since the previous evoked potential. If the process which depresses the evoked potential amplitude is cumulative, then the amount of evoked potential depression (habituation) will increase as the rate of stimulation is increased. This will be referred to later as the intrinsic inhibitory mechanism theory of AEPH. The data of this experiment are in agreement with such a theoretical position.

This theoretical position, which is consistent with the data of this experiment, will be considered again later in the dissertation.

3.4 Experiment 4 - Repeated Sessions with Loudspeakers

3.4.1 Aim

The aim of this experiment was to look at the reliability of the decrement obtained with repeated stimulation. One of the criteria for BRH is that habituation develops faster as more habituation sessions are given (1.1.1.4). Since some of the animals from Experiment 3 were used in this experiment, a powerful test of this hypothesis is not possible as these animals have been repeatedly stimulated. Instead, the reliability of the decrement obtained in three consecutive sessions of the same stimulus condition was examined. It was also decided that the spatial criteria

controlling for acoustic variability would not be applied. This would allow some estimate of the effect of this factor on the variability in habituation results.

3.4.2 Subjects, Procedure and Apparatus

Four placements in each of the CN, IC and MG from Experiment 3 were tested. Each animal was stimulated for 20 minutes with an 85 db, 20 msec click given at the rate of 1/sec. Forty-eight hours later another session was given, followed by another after a similar interval.

The same apparatus and method of scoring the changes in evoked response amplitude were employed. It was also decided not to analyse the data statistically, but to inspect the graphs for each nucleus, since the lack of control of acoustic variability would make it difficult to carry out an analysis.

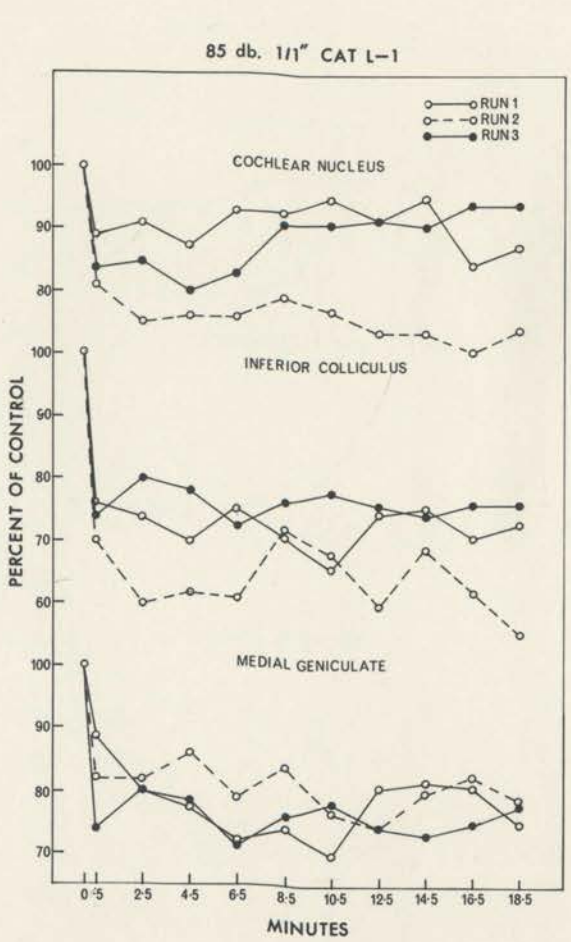
3.4.3 Results and Discussion

Figures 3-11 and 3-12 show the results for four cats covering four placements in each nucleus. It can be seen that the results for the IC and the MG are fairly reliable in that in most cases there was a similar decrement. There is no obvious evidence that the third session leads to greater habituation in these nuclei, but as stated above this is not a rigorous test of this hypothesis. The most variation occurred in the CN. In two cats L-8 and L-5, there were small increments in one session. These increments appeared to be associated with continued changes in the animal's position in the test box. This increment did not seem to be reflected in the higher centres. The CN results show variations similar to those reported by Marsh and Worden (1963).

The CN results for cat L-3 are interesting. Halfway through session 2 the animal sat up so that his head was closer to the loudspeakers. This was reflected in an increase in the CN amplitude. There was not a concurrent increase in the higher centres. This supports a suggestion that lower centres with unilateral input are more prone to reflect marked changes in the acoustic field.

Figure 3-11

Percentage of control data for two animals plotted as a function of time for three consecutive habituation sessions. The stimulus was an 85 db click given at a rate of one per second.


85 db. 1/1" CAT L–1
RUN 1
RUN 2
RUN 3
COCHLEAR NUCLEUS
INFERIOR COLLICULUS
MEDIAL GENICULATE
PERCENT OF CONTROL
MINUTES
0 ·5 2·5 4·5 6·5 8·5 10·5 12·5 14·5 16·5 18·5

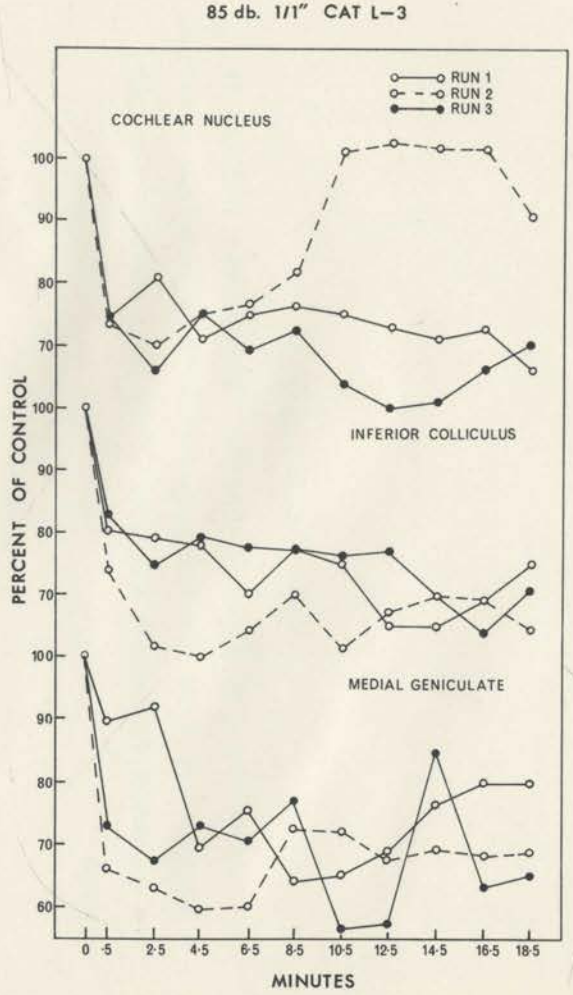
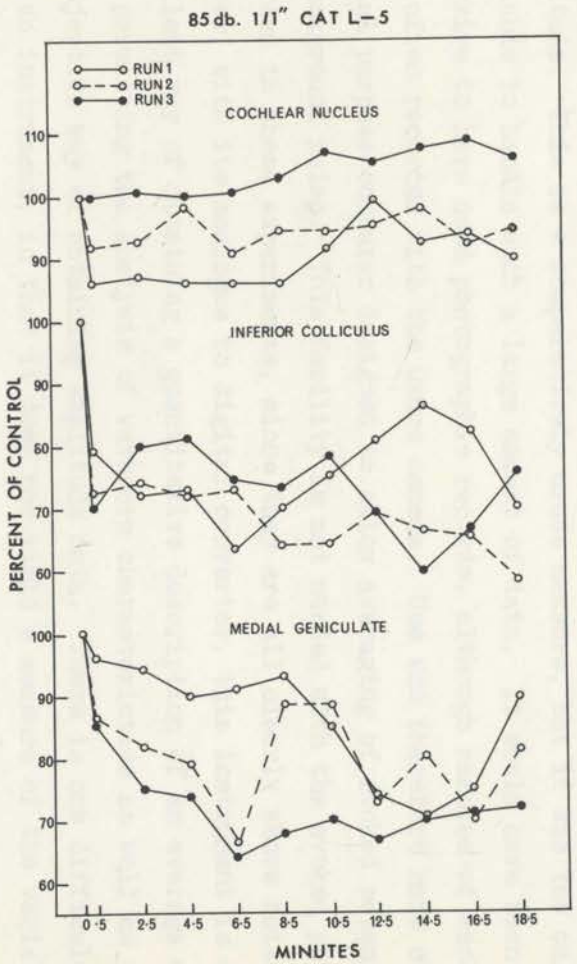
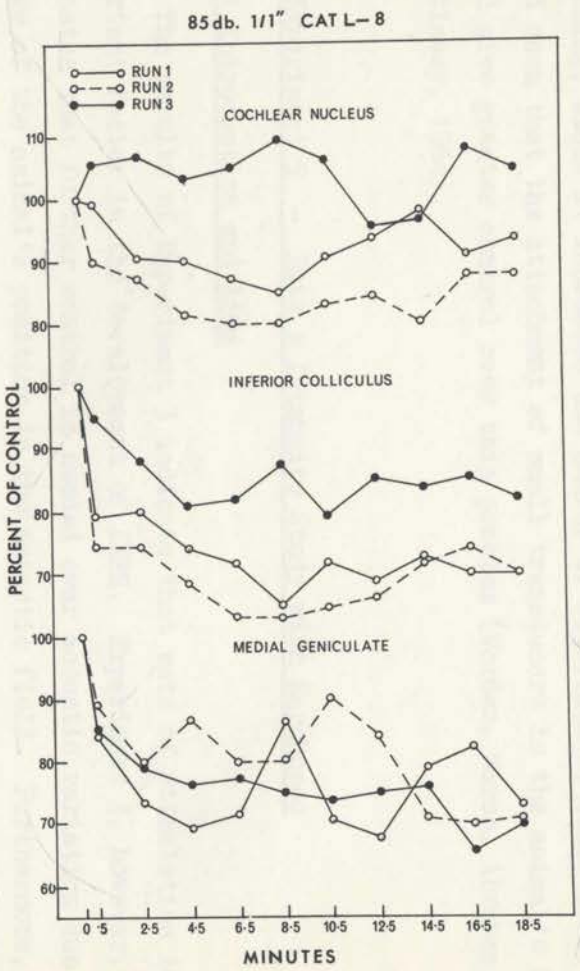

85 db. 1/1" CAT L–3
RUN 1
RUN 2
RUN 3
COCHLEAR NUCLEUS
INFERIOR COLLICULUS
MEDIAL GENICULATE
PERCENT OF CONTROL
MINUTES
0 ·5 2·5 4·5 6·5 8·5 10·5 12·5 14·5 16·5 18·5

Figure 3-12

Percentage of control data for two animals plotted as a function of time for three consecutive habituation sessions. The stimulus was an 85 db click given at a rate of one per second.


85 db. 1/1" CAT L–5
RUN 1
RUN 2
RUN 3
COCHLEAR NUCLEUS
INFERIOR COLLICULUS
MEDIAL GENICULATE
PERCENT OF CONTROL
MINUTES
0·5
2·5
4·5
6·5
8·5
10·5
12·5
14·5
16·5
18·5


85 db. 1/1" CAT L–8
RUN 1
RUN 2
RUN 3
COCHLEAR NUCLEUS
INFERIOR COLLICULUS
MEDIAL GENICULATE
PERCENT OF CONTROL
MINUTES
0·5
2·5
4·5
6·5
8·5
10·5
12·5
14·5
16·5
18·5

These results indicate that the method of controlling acoustic variability by setting spatial criteria for the position of the animal relative to the transducers is fairly effective. Under these criteria, the two sessions, in which the animal continually changed position in the box, would have been rejected. This procedure can be, of course, extremely time-consuming, since it increases the number of experimental conditions. It would seem that the attachment of small transducers to the animal's head would give greater control over this problem (Worden, Marsh, Abraham and Whittlesey, 1964).

3.5 Experiment 5 - Rate x Intensity Study with Earphones

3.5.1 Introduction and Aims

The results of Experiment 3 indicate that rate of stimulation is an important factor in the development of AEPH. Experiment 4, however, indicates that further control is needed over acoustic variation due to change of the animal's position in the acoustic field. Furthermore, the data of these experiments were based on ruler measurements of evoked potential amplitude. This is a comparatively crude measure, but it was the only one available to handle such a large amount of data. It would have been too expensive to have used photographic records, although samples of each run were often recorded with the Grass camera. Use was therefore made of a small special purpose computer designed to allow averaging of evoked potentials out of background noise. This facility is not needed with the evoked potentials recorded in these experiments, since they are all clearly above noise level. However, with its analogue to digital converter, this instrument is an excellent way of obtaining a quantitative description of an average waveform, thus permitting the analysis of waveform characteristics as well as providing an objective way of obtaining amplitude data. There is one difficulty with such an instrument, in that it does not yield a measure of the variability of amplitudes about their average value. This would require an F.M. tape recorder and an interface with general purpose computer facilities, which were not available. However, the same problem is present in Experiments 2 to 4, since the variation is discarded as an average of a block of responses

was taken. Thus the use of a fixed purpose computer, while not ideal, is a more objective method than techniques already used.

With the advent of special purpose computers capable of providing an average waveform, a number of workers have investigated the correlation between auditory evoked potentials and different stages of arousal (Winters, 1964, 1967; Chin et al, 1965). The studies have shown distinct changes in response waveform at the CN and the MG when different states of arousal are monitored. Some of the changes appear to be larger than the changes observed in Experiments 2 to 4 in response to repetitive stimulation. This suggests that such effects should be closely examined under our experimental conditions.

This experiment was designed with three aims in view:

(1) to replicate part of Experiment 3 with direct control over the acoustic field by using small earphones attached to the head of the animal, thus ensuring that the source of sound remained at a constant distance from the external ear.

(2) to use a more sophisticated method of measuring the evoked potential, namely, by means of a small fixed purpose computer. This permitted the analysis of the various components of the evoked potential.

(3) to monitor the state of arousal of the animals and to see if this were an important variable contributing toward the habituation decrement.

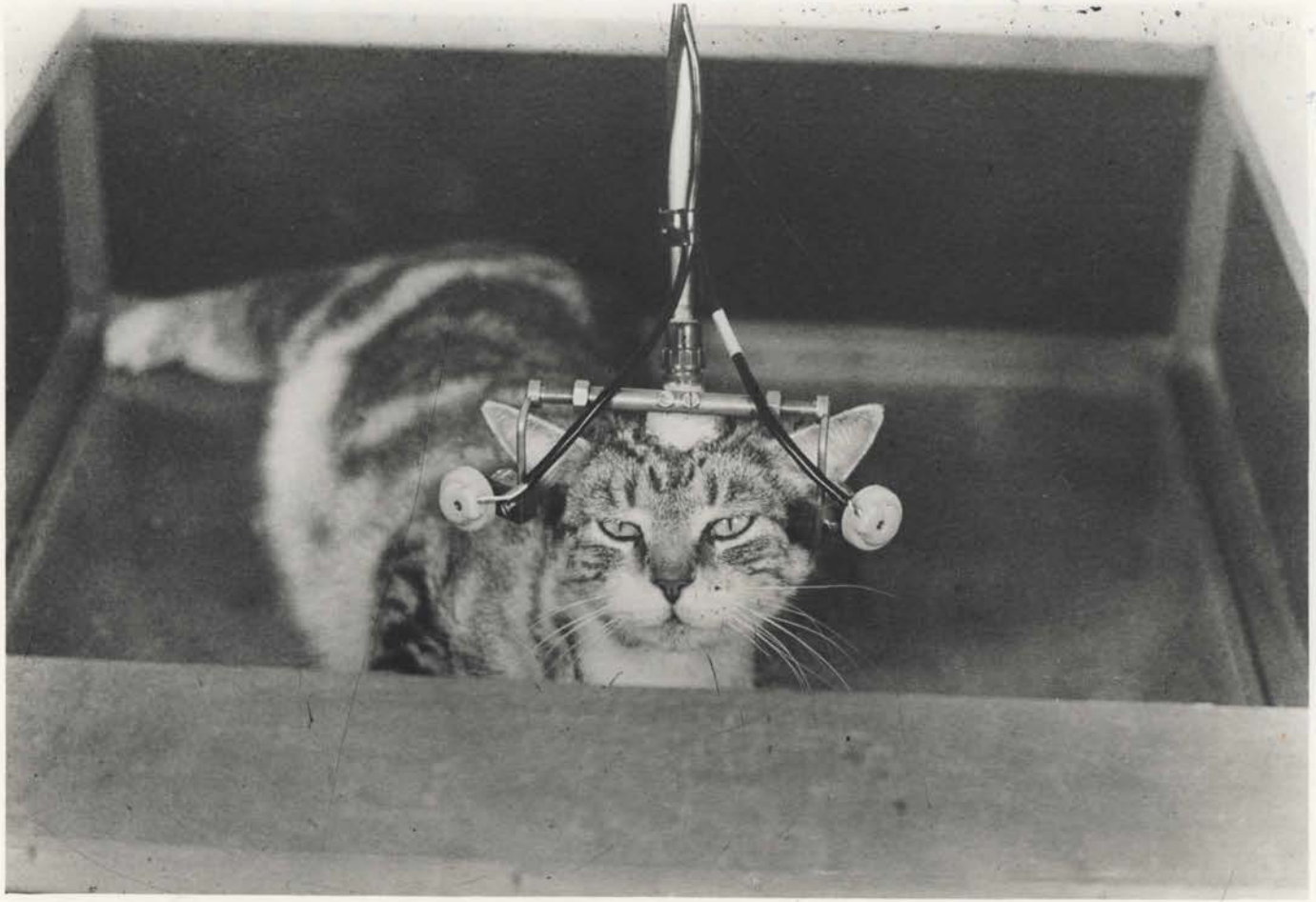
Only the IC and the MG were studied as these had shown by far the most consistent and the greatest decrement in the amplitude of responses to repetitive stimuli.

3.5.2 Subjects, Electrode Implantation and Histology

Bipolar stainless steel electrodes were implanted in ten cats in the MG (A5.0, L11.0, H0.0) and the IC (P1.5, R5.0, H2.0), using methods already outlined in 3.2.2 and 3.3.2. Similar criteria for latency and signal-to-noise ratio were applied. After recovery from the operation, seven IC and six MG placements were accepted for further analysis. In addition, two cortical screw electrodes were implanted over the auditory cortex. All

Figure 3-13

One of the experimental animals with small earphones attached to a bracket mounted on the skull.



placements were verified histologically using methods described in section 2.4.5. Figures 3-14 and 3-15 show approximate location of the electrodes in each nucleus.

3.5.3 Stimulus Parameters and Method of Delivery of Stimuli

The auditory stimulus was once again a 20 msec "click" generated as described in 3.2.3. Two rates of stimulation, 1/1 sec and 1/4 sec, and two intensities, 85 and 105 db re .0002 microbar, were chosen. The intensities were measured using a General Radio impact noise analyser (type 1556-A) in conjunction with a General Radio sound level meter (type 1551-B). These measurements were taken with a condenser microphone placed two inches from the small earphones that were to be mounted on the animal's head. Each earphone was calibrated separately so that pairs of matched earphones were obtained. This distance of two inches approximated the distance from the earphone to the cat's head in all cases. Figure 3-13 shows an animal with the earphones mounted on its head.

3.5.4 Recording of Responses

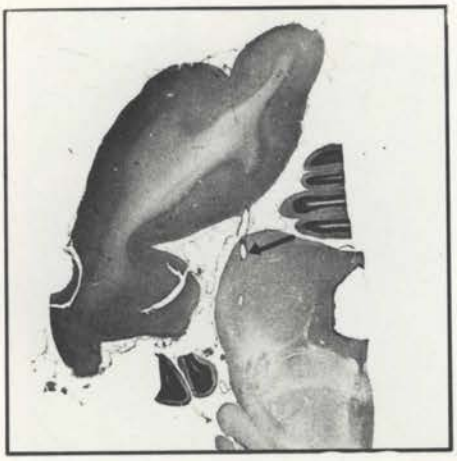
All bipolar responses were input to Tektronix 122 pre-amplifiers and automatically recorded on an Offner EEG system. The responses were also recorded on a Mnemotron Computer of Average Transients (C.A.T.) with an analysis time of 250 msec. The C.A.T. was employed, not for averaging a signal out of noise, but so as to provide a digital record of the response. The contents of the memory of the C.A.T. were output via a printer (which gave a record of each bin number and the digital number stored there), and also were often displayed on an X-Y plotter.

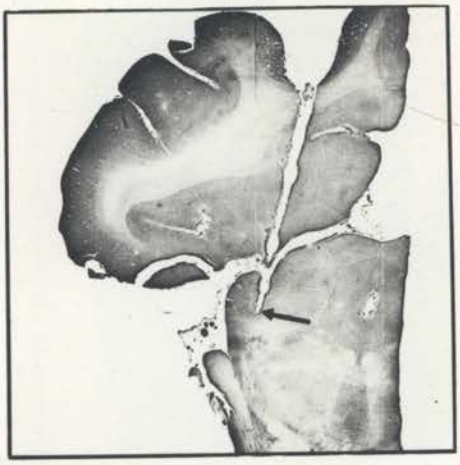
3.5.5 Test Environment, Data Collection and Design

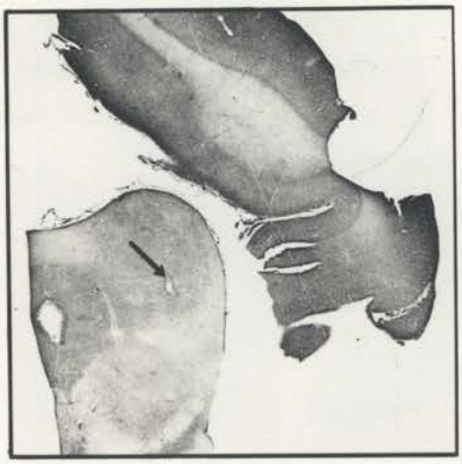
The same test box was used and each animal was placed in the box for several periods of one to two hours to accustom it to the experimental situation. Each animal was stimulated with each stimulus condition for 20 minutes, or four sessions in all. A different random order of stimulation was used for each cat. Fifty responses were averaged on the C.A.T. so that the middle of each group of responses fell at the five minute point and at

Figure 3-14

Photographs of serial sections through the inferior colliculus of six cats used in Experiment 5. Arrowheads indicate the approximate location of a lesion made through an electrode tip.







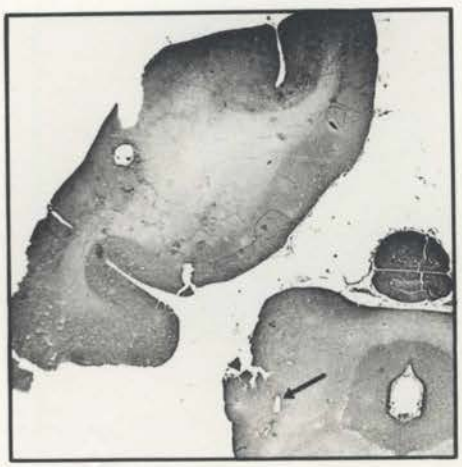
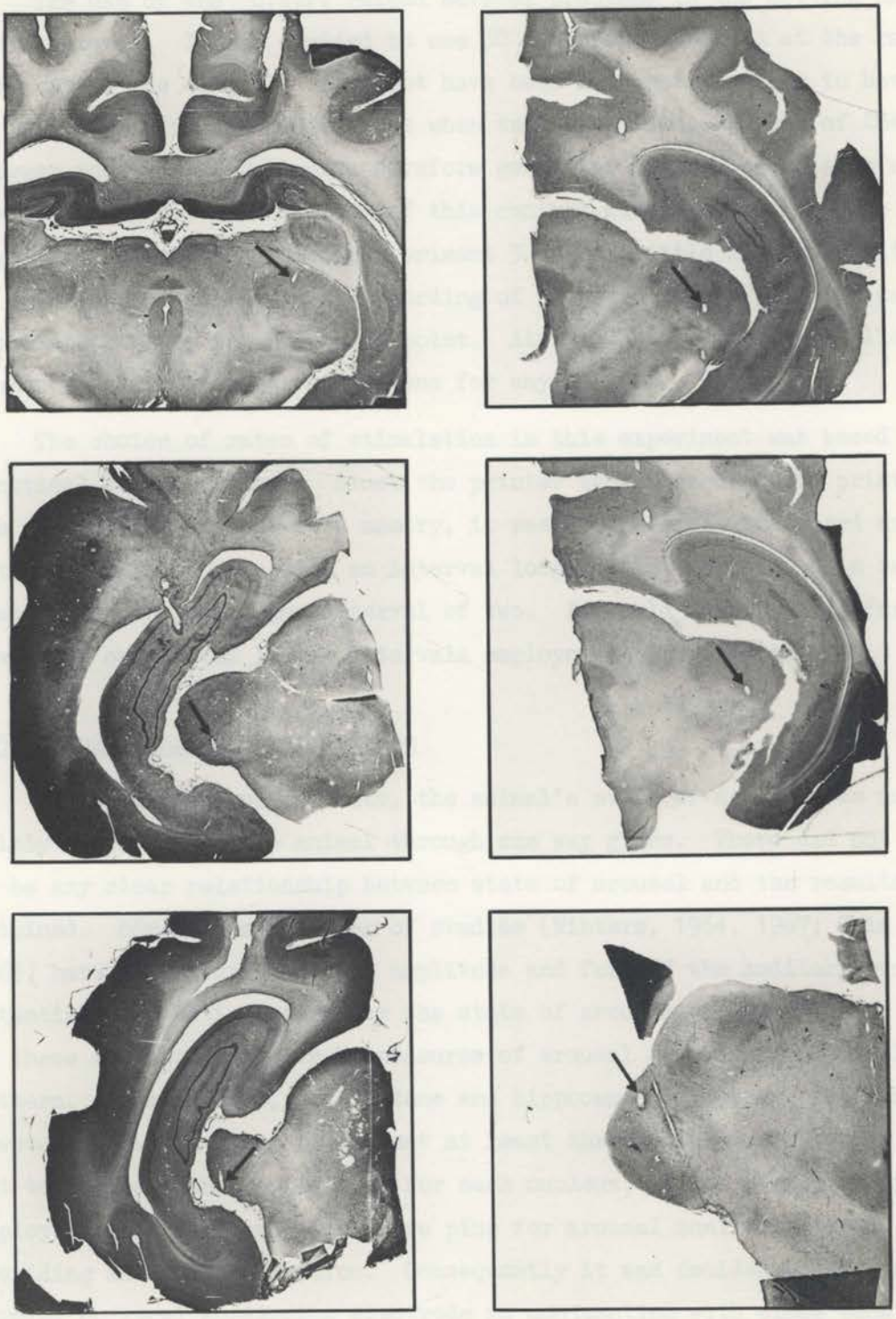


Figure 3-15

Photographs of serial sections through the medial geniculate of six cats used in Experiment 5. Arrowheads indicate the approximate location of a lesion made through an electrode tip.



each subsequent five minute period. Just before the 25 minute recording was taken, the animal was aroused and an aroused record was obtained.

The use of the C.A.T. raised several problems in the setting of a control level. It was decided to use 50 responses recorded at the rate of 1/10 sec as the control. It might have been more satisfactory to have used 50 responses given at random, but when this was tried, the use of the manual trigger mode on the Tektronix waveform generator introduced an artefact into the recording system. The use of this control was not thought to be important, as the results of Experiment 3 showed little decrement with rates of 1/10 sec. A post-control recording of 50 responses at 1/10 sec was taken immediately after the 25 minute point. At least seven days were allowed to elapse between consecutive sessions for any one cat.

The choice of rates of stimulation in this experiment was based on practical considerations. Since the printer took 90 seconds to print out the contents of the C.A.T.'s memory, it was not possible to record and print out every five minutes with an interval longer than 1/4 sec. This rate was then chosen as the longer interval of two. It would have been preferable to have used one of the longer intervals employed in Experiment 3.

3.5.6 Monitoring of Arousal State

In the earlier experiments, the animal's state of arousal was monitored solely by observing the animal through one way glass. There did not appear to be any clear relationship between state of arousal and the results obtained. Since then a number of studies (Winters, 1964, 1967; Chin et al, 1965) have shown that both the amplitude and form of the auditory evoked potentials can be influenced by the state of arousal of the animal. Often in these studies, a number of measures of arousal are taken: cortical EEG pattern, eye movements, muscle tone and hippocampal discharge pattern. However, it was desired to implant at least three bipolar electrodes in each cat to increase the sample size for each nucleus. Since a nine pin plug was employed, this left only two spare pins for arousal monitoring after providing an earth connection. Consequently it was decided to use only a bipolar cortical monitoring electrode in conjunction with close behavioural monitoring. It was realised that this does not give optimal discrimination

between arousal states. Four categories were set up for determining state of arousal:

(1) awake, eyes open and activated cortical EEG record,
(2) drowsy, eyes closed, patches of high voltage spindling on EEG cortical record,
(3) asleep, eyes closed, consistent high voltage spindling cortical EEG record,
(4) asleep, eyes closed, twitching of neck and other muscles and fast low voltage cortical EEG record (paradoxical sleep).

In the protocol for each experiment the arousal state of each animal during a recording period was noted. When each animal was aroused at the 25 minute mark, it was by turning the wing nuts on the door of the box. This awoke the animal but there was usually no movement present when the aroused recording was taken.

3.5.7 Data Reduction

The printer output of the C.A.T. memory was punched onto IBM computer cards. These data were read into a CDC 3200 and the data analysed to give two components of the averaged response, the peak-to-peak amplitude, which included the primary and the secondary deflection, and the area under the slow wave which followed the peak-to-peak deflection. The latter calculation was based on Simpson's rule (Burington, 1949, p.13). Figure 3-16 illustrates these components in the IC and the MG. Both these measures were then used in the statistical analysis.

3.5.8 Statistical Predictions and Analysis

On the basis of Experiment 3, it was decided to make separate predictions for each nucleus, but before doing so a brief outline of the basis for planned contrasts (Hays, 1963; Rodger, 1965) will be given. If the number of experimental groups be denoted by a, then the maximum number of planned contrasts which may be predicted independently of the data is $(a-1)$. The mean square for each contrast is divided by the mean square for error, and the obtained F is based on a numerator degrees of freedom of one and a denominator degrees of freedom of those of the mean square for error.

When using this method the overall F ratio is not tested.

The reason why only (a-1) orthogonal contrasts may be tested using this method is because, with independent sample means, there can only be at most (a-1) contrasts which are independent of one another and of the grand mean. It is permissible, however, to test one additional hypothesis which is not a contrast, but which has the form $H:\mu_i = Y$, where Y is some value predicted before the experiment. The general form of a contrast across a population means is

$$H_o : C_{i1}\mu_1 + C_{i2}\mu_2 + \text{---} + C_{ia}\mu_a + d_i = 0$$

where C_{ij} is a contrast coefficient and d_i is an additive constant (Rodger, 1967). The alternative to the hypothesis is

$$H_1 : C_{i1}\mu_1 + C_{i2}\mu_2 + \text{---} + C_{ia}\mu_a + d_i = \delta_i$$

where δ_i is the departure of H_1 from H_o in the population; δ_i must be expressed in units of σ, the population standard deviation.

3.5.8.1 Inferior Colliculus Predictions

The following figure sets out the means in this design to assist the statement of the planned contrasts. The scores in each cell can be either trend component coefficients or percentage means.

		Rate	
		1/1	1/4
Intensity	85	μ_1	μ_2
	105	μ_3	μ_4

The following three null hypotheses were set up for the test of trend. The three contrasts outlined are mutually orthogonal.

(1) that there would be no rate effect

$$H_o : \mu_1 + \mu_3 - \mu_2 - \mu_4 = 0$$

(2) that there would be no intensity effect

$$H_o : \mu_1 + \mu_2 - \mu_3 - \mu_4 = 0$$

This effect was investigated despite the fact that an intensity effect did not occur in Experiment 3, because it was possible that the improved stimulus control and the more efficient method of measuring the response would allow an intensity effect to appear.

(3) that there would be no interaction between rate and intensity

$$H_o : \mu_1 + \mu_4 - \mu_3 - \mu_2 = 0$$

These three contrasts exhaust the (a-1) degrees of freedom for orthogonal contrasts. They apply to the analysis of the data by the test of trend.

3.5.8.2 Medial Geniculate Predictions

One of the problems with the limitation of not being able to use slower rates than 1/4 sec, is that it might make it more difficult to obtain a rate effect at the MG. The results of Experiments 2 and 3 suggest that the MG shows larger decrements than the other nuclei studied. Experiment 3 suggests that rates around 1/4 to 1/5 sec may produce a larger decrement at the MG than at the IC. With these cautions in mind the same predictions were made for the MG:

(1) that there would be no rate effect

$$H_o : \mu_1 + \mu_3 - \mu_2 - \mu_4 = 0$$

(2) that there would be no intensity effect

$$H_o : \mu_1 + \mu_2 - \mu_3 - \mu_4 = 0$$

(3) that there would be no interaction between rate and intensity

$$H_o : \mu_1 + \mu_4 - \mu_3 - \mu_2 = 0$$

3.5.8.3 Statistical Analysis

Two measures were analysed, using the modified test of trend: peak-to-peak amplitude and area under the slow wave. Similar steps were taken to those specified in section 3.2.6 for transforming the data, and once again the test was robust to lack of homogeneity of variance. Type I and Type II error rates of .05 were set.

3.5.9.1 Nature of Evoked Activity

Figure 3-16 illustrates the type of averaged response recorded by means of the C.A.T. The peak-to-peak amplitude is shown, as is the area under the curve that is measured. The peak-to-peak deflection measured consisted of the maximum deflection in the averaged waveform and included both the primary and the secondary deflection. This procedure was felt to give a measure that was more comparable to the amplitude measures used in the earlier experiments. Since bipolar recordings were used, it was not possible to specify the polarity of the various deflections.

3.5.9.2 Inferior Colliculus

(a) Amplitude Measures

The results of the predicted contrasts for amplitude means show that there is an effect due to rate of stimulation ($p < .01$) but that neither the intensity effect nor the rate by intensity interaction are significant (Table 10).

There is a significant rate effect in the linear component of trend ($p < .05$) but no significant effect due to intensity, and a non-significant interaction.

Similar results were obtained with the quadratic components of trend. There was a significant rate effect ($p < .01$) but neither intensity nor the interaction is significant. Figure 3-17 shows the amplitude measures plotted as a function of time. There is a clear-cut rate effect and a rapid return to control levels in the post-control period.

(b) Area Measures

Table 11 sets out the results for the area under the curve. These are similar to the amplitude results for means, linear and quadratic component. In each case there is a rate effect ($p < .05$), but neither the intensity factor nor the rate by intensity interaction is significant.

These results are identical to the results of Experiment 3. There is a clear-cut decrement in both amplitude and area measures over time. The group curves approximate a negative exponential. There is a marked rate

Figure 3-16

Averaged evoked potentials recorded at the IC (A) and MG (B) showing both the peak-to-peak measurement (P/P) and the area under the slow wave (hatched area).

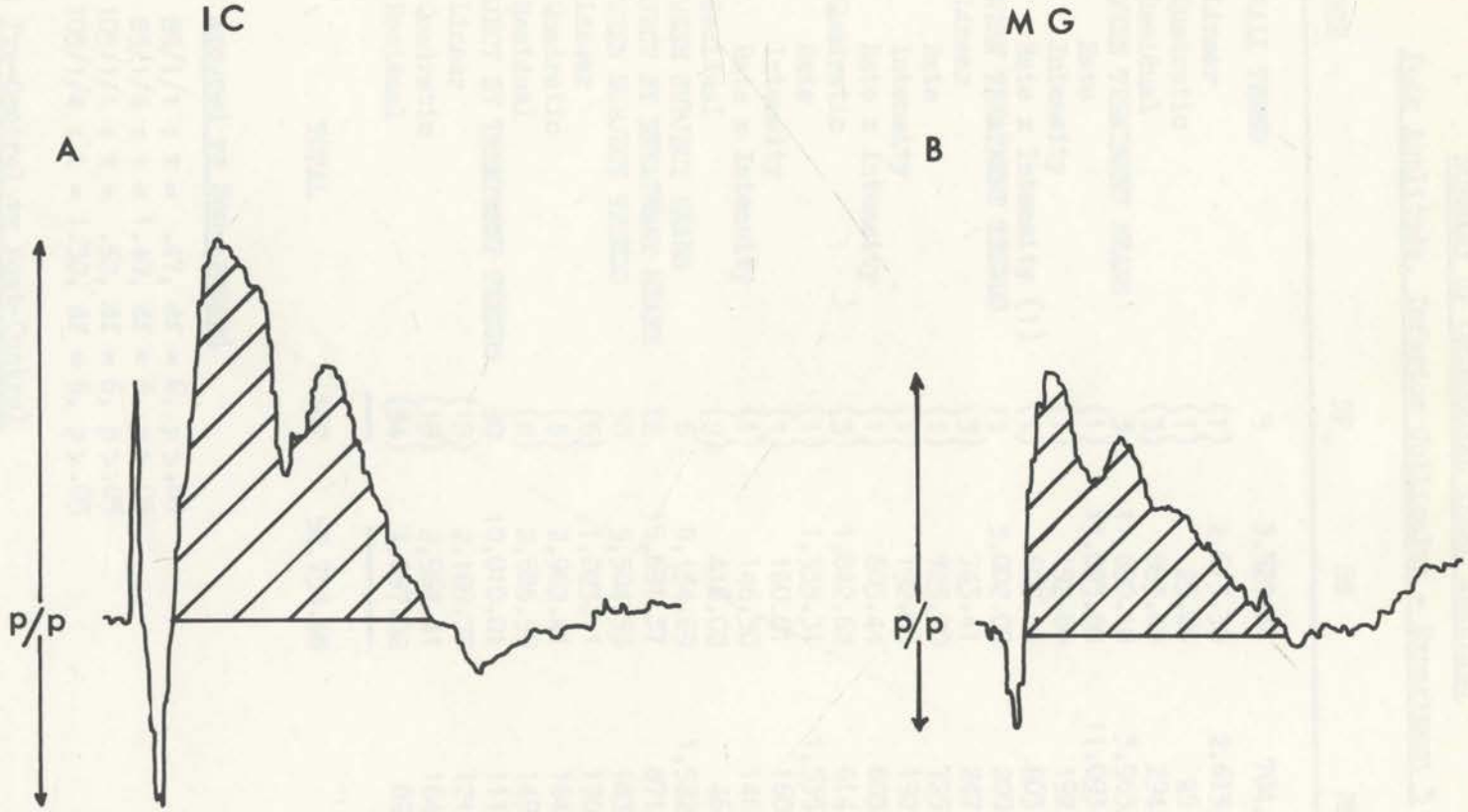

IC
A
p/p
MG
B
p/p

TABLE 10

(a) SUMMARY OF ORTHOGONAL TREND ANALYSIS

Peak Amplitude, Inferior Colliculus - Experiment 5

SOURCE	DF	SS	MS	F
OVERALL TREND	5	3,521.84	704.40	3.84 **
Linear	(1)	2,613.30	2,613,30	15.33 **
Quadratic	(1)	25.06	25.06	.08
Residual	(3)	883.48	294.49	1.91
BETWEEN TREATMENT MEANS	3	11,890.18	3,963.89	4.55 *
Rate	(1)	11,093.88	11,093.88	12.73 **
Intensity	(1)	192.86	192.86	.22
Rate x Intensity (1)	(1)	603.44	603.44	.69
BETWEEN TREATMENT TRENDS	15	3,002.05	200.14	1.80
Linear	(3)	743.41	247.80	2.04
Rate	(1)	725.90	725.90	6.00 *
Intensity	(1)	192.86	192.86	.22
Rate x Intensity	(1)	603.44	603.44	.69
Quadratic	(3)	1,842.62	614.21	3.73 *
Rate	(1)	1,535.31	1,535.31	9.33 **
Intensity	(1)	160.81	160.81	.98
Rate x Intensity	(1)	146.30	146.30	.89
Residual	(9)	416.02	46.22	.51
BETWEEN SUBJECT MEANS	6	9,134.69	1,522.45	
SUBJECT BY TREATMENT MEANS	18	15,691.37	871.74	
BETWEEN SUBJECT TRENDS	30	5,504.55	183.49	
Linear	(6)	1,023.11	170.52	
Quadratic	(6)	2,962.41	164.58	
Residual	(8)	2,685.59	149.19	
SUBJECT BY TREATMENT TRENDS	90	10,010.01	111.22	
Linear	(18)	2,189.78	121.65	
Quadratic	(18)	2,962.41	164.58	
Residual	(54)	4,857.82	89.95	
TOTAL	167	58,754.68		

(b) Aroused vs Non-Aroused

85/1/1 : t = .47, df = 6, p > .05
85/1/4 : t = 1.40, df = 6, p > .05
105/1/1 : t = .52, df = 6, p > .05
105/1/4 : t = 1.30, df = 6, p > .05

(c) Pre-Control vs Post-Control

85/1/1 : t = 1.22, df = 6, p > .05
85/1/4 : t = 1.94, df = 6, p > .05
105/1/1 : t = 1.11, df = 6, p > .05
105/1/4 : t = .98, df = 6, p > .05

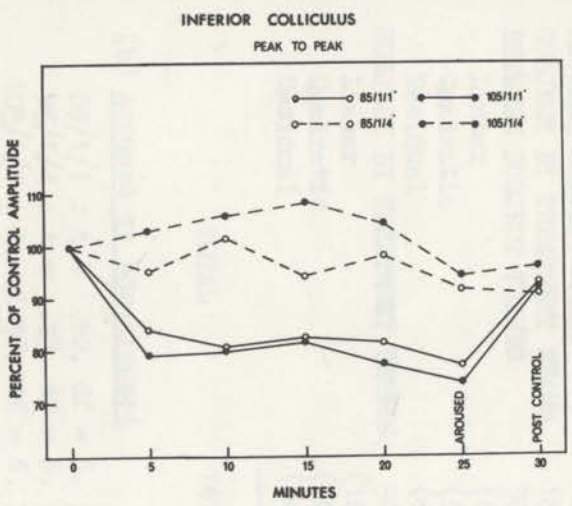
*** significant at .001 level
** significant at .01 level
* significant at .05 level

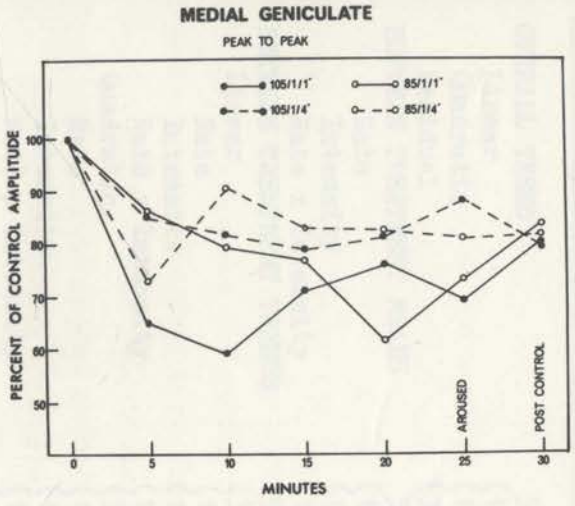
Figure 3-17

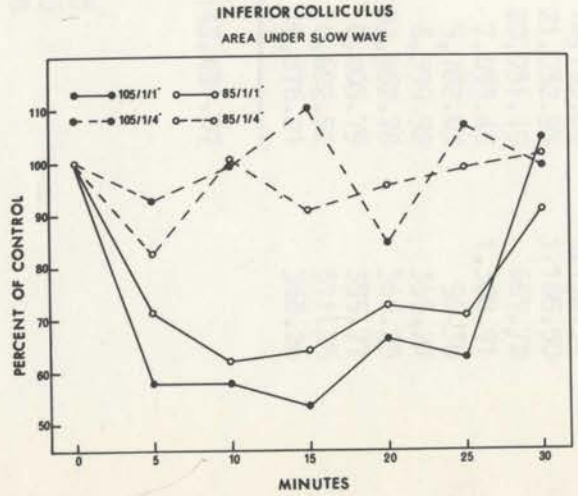
(a) The graphs in the first column show the percentage of control data for amplitude and area under the slow wave for the inferior colliculus plotted as a function of time. The parameters are 85 and 105 db intensity and rates of one per second and one per four second.

(b) The graphs in the second column show similar data plotted for the medial geniculate.

In each graph, the animals were aroused at the 25 minute mark and then a post-control was obtained.


INFERIOR COLLICULUS
PEAK TO PEAK
85/1/1"
105/1/1"
85/1/4"
105/1/4"
PERCENT OF CONTROL AMPLITUDE
AROUSED
POST CONTROL
MINUTES


MEDIAL GENICULATE
PEAK TO PEAK
105/1/1"
85/1/1"
105/1/4"
85/1/4"
PERCENT OF CONTROL AMPLITUDE
AROUSED
POST CONTROL
MINUTES


INFERIOR COLLICULUS
AREA UNDER SLOW WAVE
105/1/1"
85/1/1"
105/1/4"
85/1/4"
PERCENT OF CONTROL
MINUTES

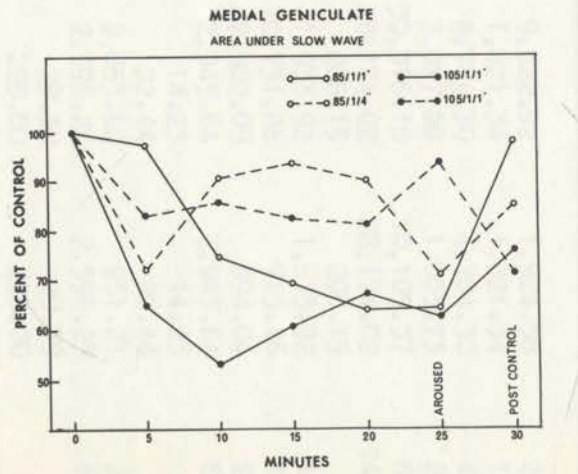

MEDIAL GENICULATE
AREA UNDER SLOW WAVE
85/1/1"
105/1/1"
85/1/4"
105/1/1"
PERCENT OF CONTROL
AROUSED
POST CONTROL
MINUTES

TABLE 11

(a) SUMMARY OF ORTHOGONAL TREND ANALYSIS

Area under slow wave - Inferior Colliculus - Experiment 5

SOURCE	DF	SS	MS	F
OVERALL TREND	5	9,622.92	1,924.58	2.88 *
Linear	(1)	1,821.34	1,821.34	1.39
Quadratic	(1)	4,784.30	4,784.30	4.96
Residual	(3)	3,017.28	1,005.73	2.82
BETWEEN TREATMENT MEANS	3	30,316.10	10,105.37	8.65 ***
Rate	(1)	28,174.02	28,174.02	24.11 ***
Intensity	(1)	208.15	208.15	.18
Rate x Intensity	(1)	1,933.92	1,933.92	1.65
BETWEEN TREATMENT TRENDS	15	10,731.86	715.46	1.95 **
Linear	(3)	2,982.09	994.04	2.79
Rate	(1)	2,945.64	2,945.64	8.28 *
Intensity	(1)	14.23	14.23	.04
Rate x Intensity	(1)	22.44	22.44	.06
Quadratic	(3)	2,853.62	951.21	2.56
Rate	(1)	2,596.23	2,596.23	6.99 *
Intensity	(1)	29.39	29.39	.08
Rate x Intensity	(1)	228.00	228.00	.61
Residual	(9)	4,896.09	544.01	1.48
BETWEEN SUBJECT MEANS	6	18,793.80	3,132.30	
SUBJECT BY TREATMENT MEANS	18	21,032.93	1,168.50	
BETWEEN SUBJECT TRENDS	30	20,031.49	667.72	
Linear	(6)	7,853.45	1,308.91	
Quadratic	(6)	5,782.63	96.38	
Residual	(8)	6,399.92	355.27	
SUBJECT BY TREATMENT TRENDS	90	32,962.78	366.25	
Linear	(18)	6,403.79	355.77	
Quadratic	(18)	6,682.52	371.25	
Residual	(54)	19,876.21	368.26	
TOTAL	167	143,491.87		

(b) Aroused vs Non-Aroused

85/1/1 : t = .62, df = 6, p>.05
85/1/4 : t = .23, df = 6, p>.05
105/1/1 : t = 1.30, df = 6, p>.05
105/1/4 : t = 1.17, df = 6, p>.05

(c) Pre-Control vs Post-Control

85/1/1 : t = 1.69, df = 6, p>.05
85/1/4 : t = .19, df = 6, p>.05
105/1/1 : t = .53, df = 6, p>.05
105/1/4 : t = .08, df = 6, p>.05

*** significant at .001 level
** significant at .01 level
* significant at .05 level

effect, but no significant effect due to stimulus intensity. Figures 3-18 and 3-19 show records from the X-Y plotter showing habituation in some of the IC potentials.

3.5.9.3 Medial Geniculate Body

(a) Amplitude Results

Table 12 sets out the results of the test of trend for the amplitude data. There is a significant rate effect for the differences between means ($p < .05$) but neither intensity nor the interaction is significant. When the linear and quadratic components of trend are examined, then the effects of intensity, rate and their interaction are not significant. This means that overall habituation has occurred but that there is not a significant difference between the curves in their shape. There is, however, a difference between the means due to rate of stimulation. Thus the faster rates have produced a greater decrement but the shape of the curve is similar for all groups. Figure 3-17 shows the data plotted as a function of time.

(b) Area Results

Table 13 summarises the test of trend in the area data. These results show that there is not a significant rate, intensity or interaction effect for either the means or the linear and quadratic components of trend. Since there is a significant overall trend and a significant linear component of overall trend, these results indicate that habituation has taken place to both rates. However, while the faster rates tend to produce a larger decrement, there is not a significant difference between the effects of the two rates.

The results confirm the findings of Experiment 3, since there is significant habituation and the faster rate of stimulation leads to a greater decrement in the amplitude measures. In general, there is little difference between the groups in the area measurements. The curves tend to approximate to a negative exponential. Figures 3-18 and 3-19 show records from the X-Y plotter in which there is habituation of MG potentials.

Figure 3-18

Averaged evoked responses recorded from the IC and MG of one cat. In the first column are control IC response (A), response after 20 minutes stimulation at one per sec (B), response after the animal was aroused (C), and IC post-control response to stimulation at one per ten sec (D). In the second column are control MG response (E), response after 20 minutes stimulation at one per sec (F), response after arousal (G), and MG post-control response to stimulation at one per ten sec. Each response is an average of 50 evoked responses. Stimulus intensity was 85 db.

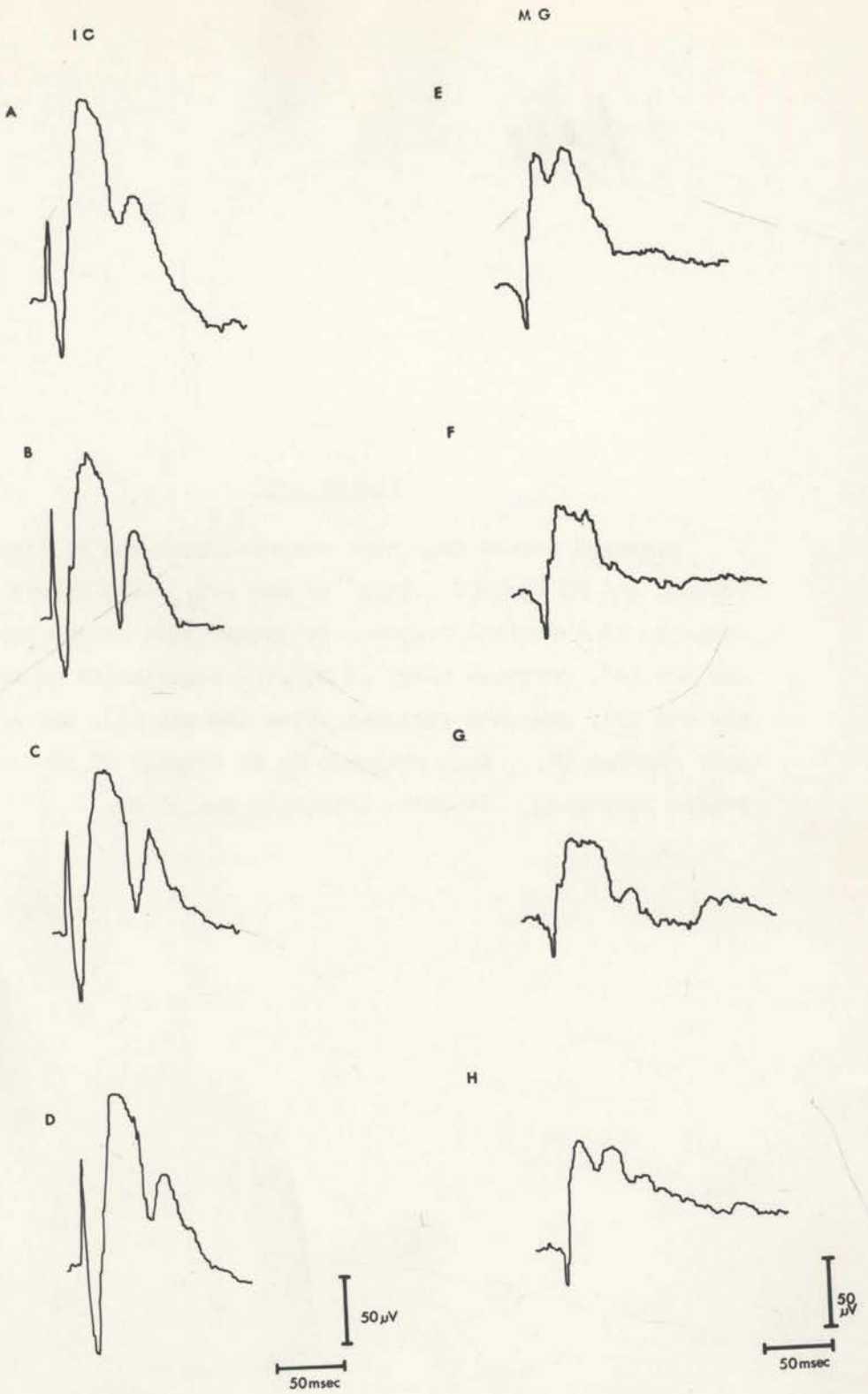

IC
MG
A
B
C
D
E
F
G
H
50 μV
50 msec
50
μV
50 msec

Figure 3-19

Averaged evoked responses recorded from the IC (first column) and MG (second column) of one cat. Each record consists of a control response to stimulation at one per ten sec (A), response after 20 minutes stimulation at one per sec (B), response recorded after arousal (C), and a post control (D). Each response is an average of 50 evoked responses. Stimulus intensity was 85 db.

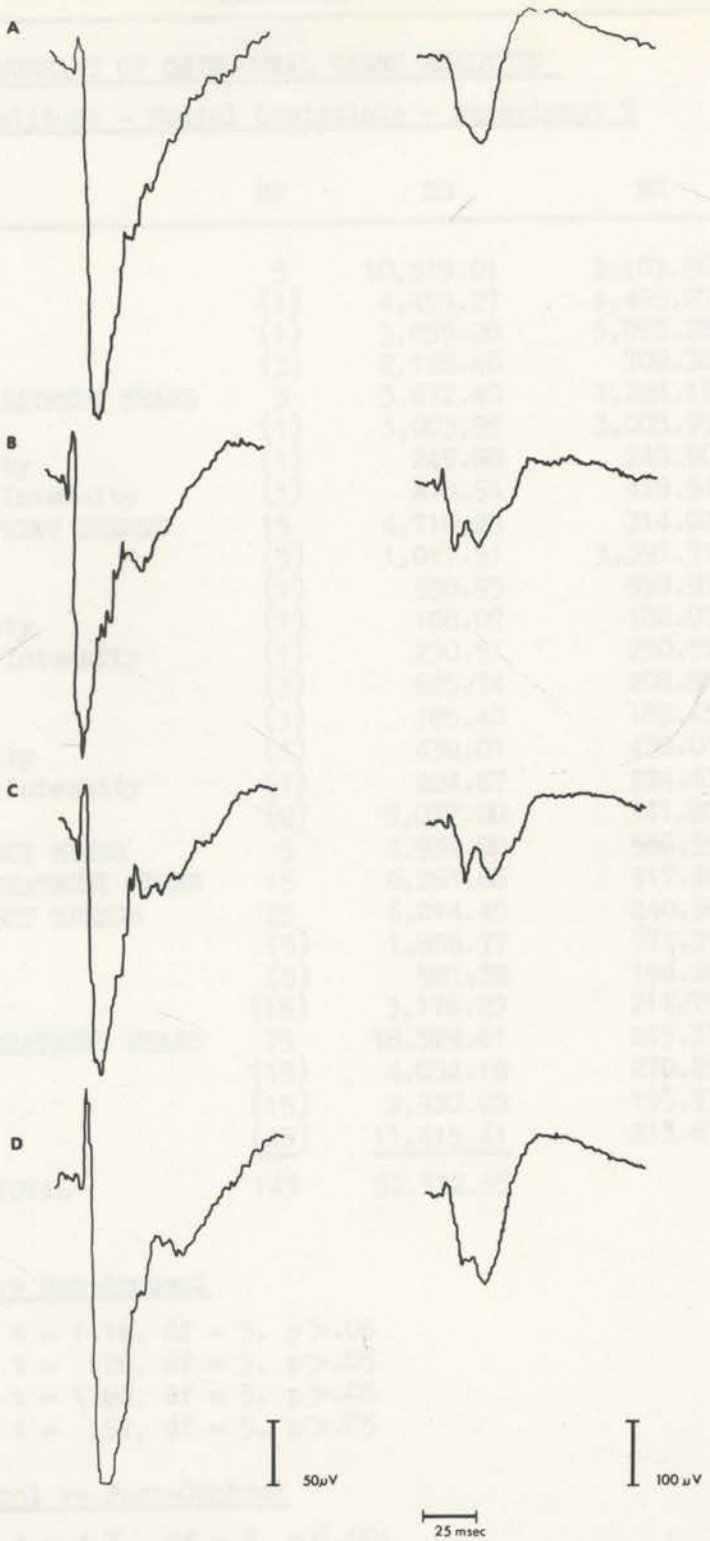

A
B
C
D
50μV
100 μV
25 msec

TABLE 12

(a) SUMMARY OF ORTHOGONAL TREND ANALYSIS

Peak Amplitude - Medial Geniculate - Experiment 5

SOURCE	DF	SS	MS	F
OVERALL TREND	5	10,519.01	2,103.80	8.74 ***
Linear	(1)	4,495.27	4,495.27	12.11 *
Quadratic	(1)	3,895.28	3,895.28	19.85 **
Residual	(3)	2,128.46	709.38	3.44
BETWEEN TREATMENT MEANS	3	3,672.40	1,224.13	2.93
Rate	(1)	3,003.95	3,003.95	7.20 *
Intensity	(1)	249.90	249.90	.60
Rate x Intensity	(1)	418.54	418.54	1.00
BETWEEN TREATMENT TRENDS	15	4,710.25	314.02	1.27
Linear	(3)	1,017.51	3,391.71	1.25
Rate	(1)	598.93	598.93	2.22
Intensity	(1)	188.07	188.07	.70
Rate x Intensity	(1)	230.51	230.51	.85
Quadratic	(3)	625.74	208.58	1.07
Rate	(1)	185.48	185.45	.95
Intensity	(1)	438.01	438.01	2.24
Rate x Intensity	(1)	224.67	224.67	.01
Residual	(9)	5,077.00	341.88	1.34
BETWEEN SUBJECT MEANS	5	2,934.98	586.99	
SUBJECT BY TREATMENT MEANS	15	6,261.86	417.46	
BETWEEN SUBJECT TRENDS	25	6,014.45	240.58	
Linear	(5)	1,856.77	371.35	
Quadratic	(5)	981.39	196.28	
Residual	(15)	3,176.29	211.75	
SUBJECT BY TREATMENT MEANS	75	18,399.61	245.33	
Linear	(15)	4,054.18	270.28	
Quadratic	(15)	2,930.02	195.33	
Residual	(45)	11,415.41	213.67	
TOTAL	143	52,512.55		

(b) Aroused vs Non-Aroused

85/1/1 : $t = 1.14$, $df = 5$, $p > .05$
85/1/4 : $t = .26$, $df = 5$, $p > .05$
105/1/1 : $t = 1.50$, $df = 5$, $p > .05$
105/1/4 : $t = .57$, $df = 5$, $p > .05$

(c) Pre-Control vs Post-Control

85/1/1 : $t = 4.7$, $df = 5$, $p < .001$
85/1/4 : $t = 2.8$, $df = 5$, $p < .05$
105/1/1 : $t = 2.58$, $df = 5$, $p < .05$
105/1/4 : $t = 2.69$, $df = 5$, $p < .05$

*** significant at .001 level
** significant at .01 level
* significant at .05 level

TABLE 13

(a) SUMMARY OF ORTHOGONAL TREND ANALYSIS

Area under curve - Medial Geniculate - Experiment 5

SOURCE	DF	SS	MS	F
OVERALL TREND	5	10,420.28	2,084.06	3.74 *
Linear	(1)	4,084.39	4,084.39	13.34 *
Quadratic	(1)	5,163.20	5,163.20	5.54
Residual	(3)	1,172.69	390.89	18.77 ***
BETWEEN TREATMENT MEANS	3	5,350.88	1,783.63	.77
Rate	(1)	4,803.65	4,803.65	2.08
Intensity	(1)	109.38	109.38	.05
Rate x Intensity	(1)	378.86	378.86	.19
BETWEEN TREATMENT TRENDS	15	13,809.63	920.64	1.20
Linear	(3)	3,725.84	1,241.95	1.31
Rate	(1)	676.78	676.78	.71
Intensity	(1)	2,053.75	2,053.75	2.16
Rate x Intensity	(1)	995.30	995.30	1.05
Quadratic	(3)	6,551.02	2,183.67	2.85
Rate	(1)	3,155.77	3,155.77	4.11
Intensity	(1)	3,098.77	3,098.77	4.04
Rate x Intensity	(1)	296.47	296.47	.39
Residual	(9)	3,532.77	392.53	.99
BETWEEN SUBJECT MEANS	5	34,302.56	6,860.51	
SUBJECT BY TREATMENT MEANS	15	34,664.25	2,310.95	
BETWEEN SUBJECT TRENDS	25	13,949.23	557.97	
Linear	(6)	7,853.45	1,308.91	
Quadratic	(6)	5,782.63	963.77	
Residual	(13)	313.15	20.87	
SUBJECT BY TREATMENT TRENDS	90	32,962.78	366.25	
Linear	(18)	6,403.79	355.77	
Quadratic	(18)	6,682.52	371.25	
Residual	(54)	19,876.47	441.70	
TOTAL	167	143,491.87		

(b) Aroused vs Non-Aroused

85/1/1 : t = .03, df = 5, p > .05
85/1/4 : t = 1.85, df = 5, p > .05
105/1/1 : t = .84, df = 5, p > .05
105/1/4 : t = 1.43, df = 8, p > .05

(c) Pre-Control vs Post-Control

85/1/1 : t = .09, df = 5, p > .05
85/1/4 : t = 1.66, df = 5, p > .05
105/1/1 : t = 3.22, df = 5, p < .05
105/1/4 : t = 2.73, df = 5, p < .05

*** significant at .001 level
** significant at .01 level
* significant at .05 level

3.5.9.4 The Effects of Arousal

Figure 3-17 shows clearly that evoked potentials recorded after the animal is aroused do not differ from evoked potentials recorded when the animal is not aroused (Figs. 3-18 and 3-19). The results of the t tests for both the amplitude and area data were not significant (Tables 10b, 11b, 12b, 13b). It would appear that AEPH decrements are not determined by the state of arousal of the animal.

However, the results are not as simple as the above analysis would suggest. Since each test session was quite brief, many of the cats remained awake. When sleep did occur, it was not often that all four arousal states were observed. In particular, paradoxical or REM (rapid eye movement) sleep did not occur very often, but on these occasions there was a considerable decrement in both IC and MG amplitude (Figs. 3-20 and 3-21). There did not appear to be any changes in the potentials recorded during other sleep states, but one MG placement showed a consistent change during synhronised slow wave sleep. This change consisted of an increase in the size of the slow wave (Fig. 3-21), but it was not possible to specify the polarity of the change as bipolar electrodes were employed.

3.5.9.5 Pre-Control vs Post-Control

In the IC, the post-controls did not differ from the pre-controls (Tables 10c, 11c). However, there were significant differences between post-controls and pre-controls recorded at the MG (Tables 12c, 13c). This result indicates that recovery at the IC has been very rapid, and at the MG, the recovery has been much slower.

3.6 Discussion

This experiment introduced two improvements on previous experiments in that acoustic variation in the test box was controlled by using small earphones mounted on the head of each animal and more accurate measurements were obtained from the fixed purpose computer. It is clear that under these experimental conditions, the results obtained in the IC confirm Experiment 3. Both the amplitude and the area under the curve measures are

Figure 3-20

Averaged IC evoked potentials recorded from three cats during three arousal states (alert, synchronised slow wave sleep (SS sleep) and rapid eye movement sleep (REM)). Each row shows potentials recorded from one placement during the three states. There is a considerable reduction in the potentials recorded during REM sleep. Each record is the average of 50 evoked potentials.

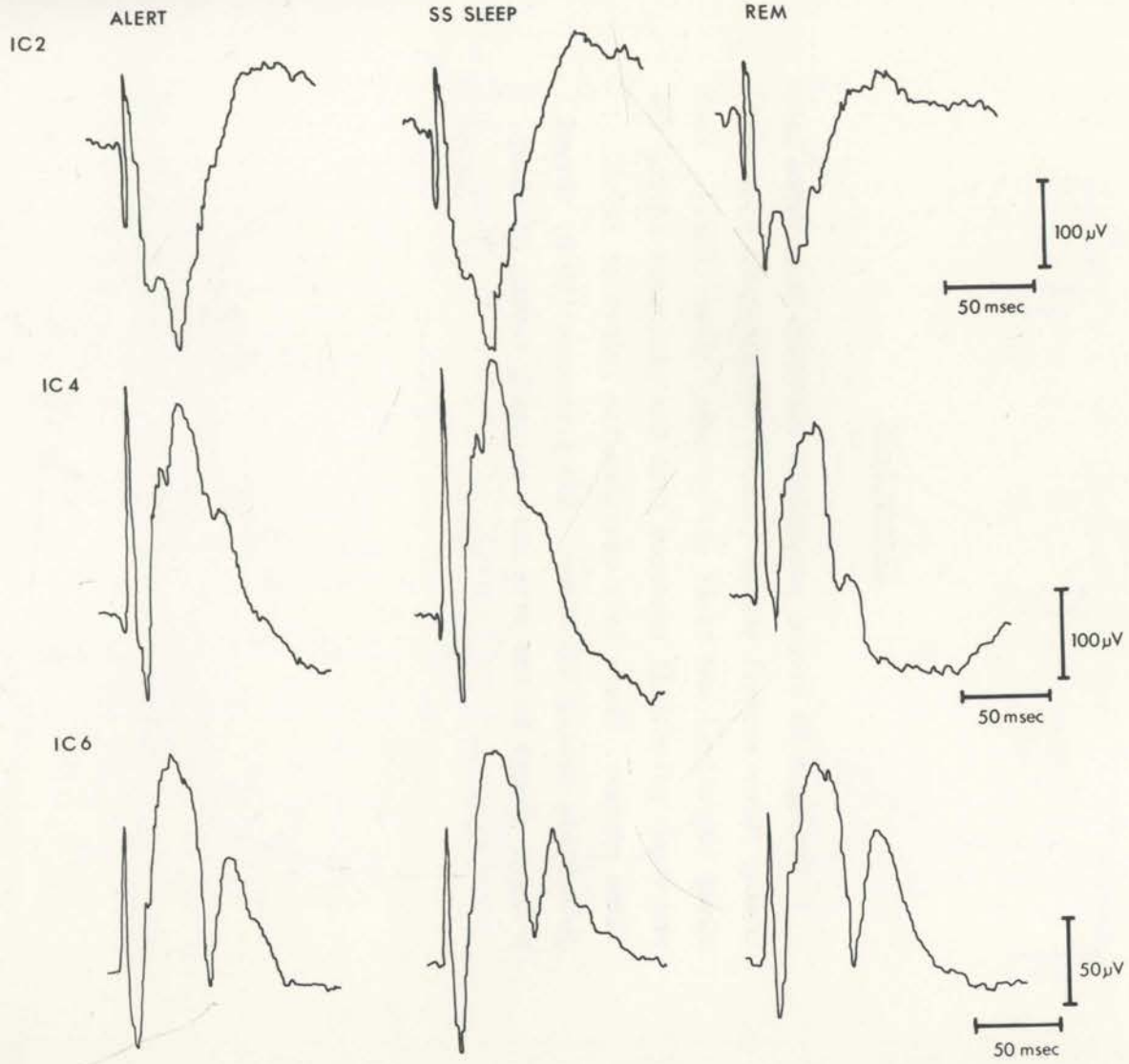
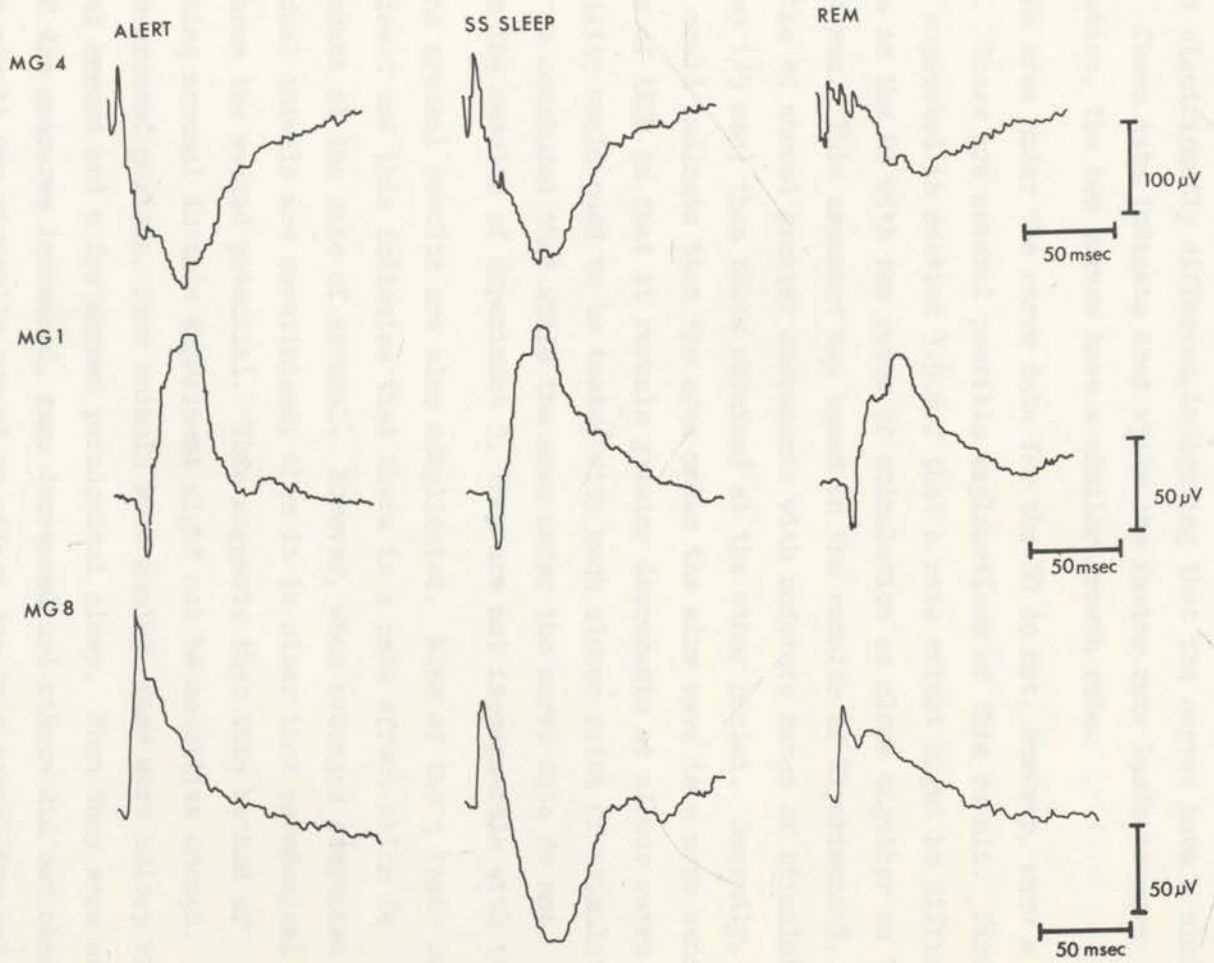

ALERT
SS SLEEP
REM
IC2
IC4
IC6
100 μV
50 msec
100 μV
50 msec
50 μV
50 msec

Figure 3-21

Averaged MG evoked potentials recorded from three cats during three arousal states (alert, synchronised slow wave sleep (SS sleep) and rapid eye movement sleep (REM)). Each row shows potentials recorded from one placement during the three states. There is a considerable reduction in all potentials during REM sleep. One placement (MG 8) showed a large change in the slow wave activity during SS sleep.


ALERT
SS SLEEP
REM
MG 4
MG 1
MG 8
100 μV
50 msec
50 μV
50 msec
50 μV
50 msec

influenced by rate of stimulation. There is no significant effect due to stimulus intensity.

The results obtained in the MG are not quite as easy to interpret. There is a difference between amplitude means due to rate of stimulation and this is what also occurred in Experiment 3. However, the components of trend are not significantly different, indicating that the curves have a similar shape. These data indicate that while the faster rate leads to more habituation, the two curves have a similar growth rate.

The area under the curve data for the MG do not, however, show a rate effect. There are several possible explanations of this result. Firstly, it was suggested in section 3.5.8.2 that a rate effect might be difficult to achieve in the MG with two rates of stimulation as close together as 1/1 sec and 1/4 sec. This argument was based on the results of Experiment 3, in which the MG showed greater decrements with moderate rates of stimulation (1/1 and 1/5 sec) than those obtained at the other nuclei. Secondly, the result could indicate that the area under the slow wave is a more sensitive measure of AEPH in that it reveals greater decrements at slower rates. This possibility would need to be tested with much slower rates of stimulation. It can be concluded that while the area under the curve data do not confirm the results of Experiment 3, they are not incompatible with them.

The arousal results are also complicated. None of the t tests were significant and this indicates that there is a rate effect which is independent of the rate of arousal. However, when averaged responses for individual animals are scrutinised, then it is clear that paradoxical sleep influences the evoked potential. This suggests that this method of evaluating arousal in this experiment might not be sensitive enough. During the non-aroused periods, some animals were dozing, some were asleep with a cortical record and a few showed paradoxical sleep. When they were aroused, some of the measures increased, some decreased and others did not change. The net result was virtually one of no effect due to a cancelling out of these various changes. In a sense, the state of arousal could be said to be a random variable with respect to the main effect of rate. Thus the strong rate effect is observed despite changes due to arousal.

These results suggest that a more adequate procedure would be to obtain separate controls for the various arousal states and these would then be compared with habituated responses under similar arousal conditions. In addition, a much longer period of stimulation would be required so that all animals would have the opportunity to go through all the stages of sleep. It is interesting to note that "paradoxical" sleep produces a marked decrement at both nuclei. It is possible that this effect is produced peripherally by the middle ear muscles. Several studies (Baust et al, 1964; Dewson et al, 1965) have indicated that phasic contractions of the middle ear muscles occur during REM, or "paradoxical" sleep. These contractions produce decrements in the round window response, and this decrement could be transmitted to higher centres. This possibility would need to be tested by concurrent recordings at the round window, CN, IC and MG. Despite these inadequacies in the design, it still seems reasonable to conclude that AEPH is influenced markedly by rate of stimulation independently of state of arousal.

3.7 General Discussion of Parametric Experiments

The experiments reported in this chapter have established the following main points:

(1) Consistent decrements occur in auditory evoked potentials at the CN, IC and the MG in response to repetitive stimulation. These decrements still occur when movement in the acoustic field is controlled indirectly or directly.

(2) The evidence for the effect of intensity is conflicting. At the CN, there appears to be no intensity effect. At the IC, the least intense stimulus produced a slightly larger decrement in Experiment 2, but this effect failed to appear in Experiments 3 and 5. At the MG, the more intense stimulus produced a greater decrement in Experiment 1, but this effect was also not confirmed in Experiments 3 and 5. It seems reasonable to conclude that stimulus intensity is not an important determinant of the decrement at each nucleus.

(3) The decrements obtained at all three nuclei are a direct function of rate of stimulation. The magnitude of the decrements is greatest at the MG,

and is quite small at the CN. The magnitude decrement at the IC is intermediate between the other two.

(4) The decrements are still present despite changes in the animal's state of arousal. It is clear that REM sleep can produce marked changes and any study of AEPH that extended over long periods would need some control over this variable.

(5) The failure to find a difference between weak and strong stimuli, when both are delivered at rapid rates of stimulation, could indicate that the observed decrements might not be a true habituation process.

While these experiments show that a reliable decrement in auditory evoked potentials can occur to repetitive stimulation with adequate controls, the evidence that relates this phenomenon to BRH is not so clear-cut. In general, the experiments in which intensity has been varied have not obtained results similar to those from studies of BRH, viz., the greatest habituation with the least intense stimulus and very little habituation with intense stimuli (section 1.1.1). It is possible that the failure to find this intensity effect is due to the limited range of intensities employed. An intensity effect was observed at the IC using intensities of 75, 85, 95 and 105 db. However, as the effects of 75 and 85 db were not significantly different and as 85 db gave a better signal-to-noise ratio, the latter intensity was used in the later experiments. This possible explanation would not explain the results obtained in Experiment 2 at the MG, where the effects were in the opposite direction to those predicted from BRH. One point should however be stressed. In all the experiments, considerable decrements were obtained with 105 db stimuli. This was the maximum level the apparatus could generate, and by analogy with BRH one would have expected little habituation. This is far more difficult to reconcile than the lack of difference in effects between 85 and 105 db stimuli.

It could also be argued that 85 db is too intense and not sufficiently different from 105 db and thus the recording was obtained at some plateau of effect of intensity. Therefore one would not expect any differential effect. If this is accepted then it would also mean that the other intense stimulus (85 db) also produces a considerable decrement. This would also be difficult to explain in terms of BRH. However, against this argument is the fact that there is a considerable difference in the amplitude of evoked potentials to

both stimuli. That is, the 105 db stimulus produces a larger evoked potential (see Figures 3-8, 3-9 and 3-10). This would not be expected if a plateau level of responding had been reached.

The observation that AEPH decrements are largely a function of rate of stimulation is not, on the surface, incompatible with data on BRH. For example, Prosser and Hunter (1936) showed a marked rate effect with BRH to auditory stimuli. Even the finding that there is no AEPH with rates of stimulation as slow as 1/20 sec is not incompatible with their work as they did not establish an upper bound for their effect. However, the failure to find a significant interaction between rate and stimulus intensity does suggest that the AEPH decrements might not represent true habituation effects.

If the decrements obtained in the above experiments are not true habituation effects, then what can they represent? A suggestion was put forward in section 3.3.8, that these data could be explained by postulating a refractory phenomenon. The finding of a continuity of effects from slower to faster rates of stimulation is suggestive of a refractory mechanism, especially when it is considered that relative refractory period effects for evoked potentials have been observed in the thalamus with intervals between stimuli as long as two to five seconds (Bishop, 1964). If one assumed that inhibitory processes are involved and that these inhibitory processes can last as long as two to five seconds and that they can accumulate with continued repetitive stimulation, then this suggestion could explain the data of the parametric experiments. (This speculation also depends on the working assumption that the decrements represent a net loss of excitation.)

The main conclusion from these experiments is that repetitive stimulation produces reliable evoked potential changes that are worth further study. It is felt that this can best be achieved in the context of various theories (including the above tentative theory) that have been put forward to explain AEPH decrements. The next chapter will outline and discuss such theories.

CHAPTER 4

4.0 Theories of Auditory Evoked Response Habituation

4.1 Introduction

The experiments described in section 3.0 have shown that consistent evoked potential decrements in both peak-to-peak amplitude and slow wave activity can be obtained in the CN, the IC and the MG. A small amount of habituation occurs at the CN, much more occurs at the IC and the greatest amount occurs at the MG. The experiments have shown that this effect is largely determined by the rate of stimulation. A number of theories have been proposed to account for AEPH, and these will be examined to determine whether empirical tests of each theory can be made. It could be argued that the following theories are more in the nature of hypotheses about AEPH, since they are not highly developed theoretical systems. However, the more general term will be used to describe these positions.

4.2 Conditioning of the Middle Ear Muscles

In sections 1.3.3 and 1.3.4, some experiments by Guzman-Flores and colleagues were considered. They reported that habituation decrements in the MG and the auditory cortex were abolished by cutting the middle ear muscles or by administering Flaxedil, which paralyses the muscles. Guzman-Flores rejected any explanation of habituation based upon tonic activity of the middle ear muscles, because the action of these muscles is too slow compared with the latency of the evoked potentials.

Before examining the explanation proposed by Guzman-Flores, an analysis of the evidence rejecting a role for the tonic activity of these muscles will be briefly made. As Guzman-Flores pointed out, the fastest latency reported for the middle ear muscles is eight to ten milliseconds (Galambos and Rupert, 1959), which is longer than the latency of subcortical areas of the auditory pathway. However, there is a more important factor to be considered. Although eight to ten milliseconds is the latency of the first signs of activity in these muscles, it takes much longer for them to contract to a stage where they can produce marked effects on transmission through the

middle ear. Wersäll (1958) has pointed this out and has calculated the half-contraction time of these muscles; it is of the order of 40 msecs. Thus it would seem that not only is the evocation latency too long, but that their major influence, when they are evoked, is far too late. There is also evidence that the response of these muscles will itself habituate if they are repeatedly stimulated (Simmons and Beatty, 1964a). For them to produce habituation decrement, they would need to increase their strength of activity over time.

Guzman-Flores proposed that the action of the middle ear muscles is brought about by classical conditioning (Alcaraz et al, 1962). He pointed out that Simmons et al (1959) have shown that the response of the middle ear muscles can be classically conditioned to a light acting as a conditioned stimulus (CS). In addition, there is also evidence that time intervals can act as a CS for a classically conditioned response (Kimble, 1961). Thus, Guzman-Flores suggests that "acoustic habituation may be viewed as a positive conditioned reflex in which the time interval between stimuli represents the conditioning stimulus. Therefore, the click stands for the unconditioned stimulus and contraction of the intrinsic ear muscles for the resulting conditioned response" (Alcaraz et al, 1962, p.5).

A necessary condition for this theory is that the stimuli should be given at regular equal intervals. Thus a test of this theory would be to stimulate an animal with a set of stimuli given with random intervals but with a mean interval value of X secs. The result of this stimulation could be compared with a set of stimuli given at regular intervals equal to the value of X. If habituation occurred with the random series, this would disprove this conditioning theory; if it did not occur this would confirm the theory.

4.3 Afferent Neuronal Inhibition

Hernández-Peón, in a series of papers (Hernández-Peón et al, 1957; Hernández-Peón, 1955; 1959; 1960), proposed that AEPH is produced by an inhibitory process arising in the reticular formation. He calls this process afferent neuronal habituation and distinguishes it from such phenomena as

habituation of the orientation reaction (Lynn, 1966), which, using the present terminology, would be an example of BRH.

Hernández-Peón's evidence for the role of the reticular formation in AEPH is as follows:

(1) Under barbiturate anaesthesia, evoked potentials remain stable and do not habituate. Since barbiturates are said to act with greater potency on complex areas such as the reticular formation (Goodman and Gilman, 1960, p.126), this evidence is taken to indicate that the action of the reticular formation has been suppressed. The experimental evidence for the effects of barbiturate on AEPH has been summarised in section 1.3, and it will be remembered that Hernández-Peón's evidence is not fully documented in his papers. However, Al'tman (1960) has reported that habituation at the round window, MG and the auditory cortex did not occur after injection of barbiturates. Al'tman used the procedure of injecting and then commencing the habituation session after the animal was anaesthetised. All that can be said of this method is that it shows that habituation will not occur if the animal has been anaesthetised before stimulation begins. It still leaves open the question of whether existing habituation is abolished after barbiturates are administered.

(2) After lesions are made in the mesencephalic tegmentum, repetitive stimulation fails to produce habituation.

(3) Electrical stimulation of the mesencephalic reticular formation depresses activity in the auditory pathway.

(4) If a novel stimulus is presented then dishabituation occurs.

(5) If the auditory stimulus is paired with a stimulus such as an electric shock, then recovery from habituation occurs.

(6) Recovery from habituation occurs after a period of rest from stimulation.

It would seem that the first two points are the critical ones for this theory. The observation about electrical stimulation of the reticular formation is not so critical, since such gross stimulation could produce changes other than in the reticular formation. The evidence concerning dishabituation, conditioning and spontaneous recovery does not bear on the

hypothesis directly, since these are characteristics of BRH. These latter observations are compatible with a disruption of inhibitory processes; but they do not show that the effects are based on inhibition or where the inhibition is located.

Thus it would appear that the strongest evidence for the theory of the afferent neuronal inhibition is the work of Hernández-Peón and Al'tman. However, it has already been pointed out in sections 1.3.1 and 1.3.3 that there are methodological problems with both sets of data. It would seem that this theory needs to be tested with more stringent experimental conditions.

4.4 Cortical Inhibition

There is a considerable literature and a number of theories concerning the role of the cortex in habituation (Lynn, 1966). These theories are of intrinsic interest but they are concerned with habituation of the orientation response. This is, of course, a molar response, and another example of BRH. For this reason, models of habituation based on the cortex and applied to the orientation response will not be considered (Sokolov, 1963; Jouvet, 1961).

However, Desmedt (1960) has put forward a specific cortical theory to account for AEPH. He pointed out that the basis for Hernández-Peón's reticular theory rests on evidence involving the effects of barbiturates and lesions. When Desmedt instead stimulated the reticular core of the brain stem of awake animals, he found that "the cochlear nucleus response to a standard sound has not been affected" (Desmedt, 1960, p.162). He did not specify exactly what his results were, but it seems that he did not obtain response depression. In contrast, he reported that stimulation of "a more laterally situated and circumscribed system" (p.162) leads to a marked suppression of the CN response. This area is not part of the reticular formation. Desmedt (1960) has found a series of areas, all very close to the classical auditory pathway, which yield suppression in the CN, but not at the round window. This suggests that the olivo-cochlear bundle is not involved. He pointed out that auditory area 1 does not have connections with this descending pathway although it has descending connections with the MG and the IC. However, the more ventrally activated temporoinsular cortex

does have connections with this area. So he proposes that AEPH is based on a centrifugal system controlled by this "associative" area of the cortex.

The evidence for this system is entirely indirect. It comes from anatomical data, from stimulation studies, and from evidence for dishabituation. The latter is compatible with most theories of AEPH. It is difficult to devise a direct test of this position. However, it might be possible to test the theory indirectly by investigating dishabituation in the present experimental context, as previous studies appear to contain methodological flaws (1.3).

4.5 Intrinsic Inhibition

The fact that AEPH varies with rate of stimulus presentation suggests that some sort of inhibitory feedback process is operating within the nucleus itself. It was suggested in section 3.3.5 that intrinsic long-lasting inhibitory mechanisms might be present in the various nuclei of the classical auditory pathway. It was further suggested that each stimulus evokes a field potential which terminates in a long-lasting (order of seconds) period of depressed excitability and, as a consequence, another stimulus occurring during this time will evoke a field potential whose reduced amplitude depends on the time elapsed since the previous evoked potential. If the process which depresses the evoked potential is cumulative, then the amount of evoked potential depression (habituation) will increase as the rate of stimulation is increased. The evidence for this theoretical position is based on the observations about the effects of rate of stimulation and on the evidence that it is possible to have long-lasting inhibitory processes (probably based on interneurones) in the central nervous system (Burke and Sefton, 1966; Bishop, 1964).

At this stage the theory makes no predictions about the effects of novel stimuli and conditioning on AEPH, but it would predict that barbiturates would increase the decrement. This prediction is based on evidence that barbiturates depress activity in the central nervous system (Marshall, 1941; Chang, 1959). This prediction is opposite to predictions which would be made by the reticular inhibitory theory. Evidence in support of the prediction

would be difficult to encompass in Desmedt's centrifugal theory, so in that sense an indirect test of this position is impossible.

This theory has similarities to one put forward by Horn (1967). He argues that habituation "can be accounted for on the hypothesis that the gradual waning of response of a system of neurones to a stimulus which is slowly and repeatedly applied is a result of self-generated depression of sensitivity (SGD) at one or more points in the system" (Horn, 1967, p.707). Horn points out that at this stage he does not want to specify the actual mechanism behind SGD and that it could include such phenomena as after hyperpolarisation, recurrent inhibition, conduction block, and synaptic depression. Most of Horn's evidence comes from studies showing that the firing of some neurones will be depressed or abolished to one repetitive stimulus and that the firing will be restored by presentation of a stimulus of another modality. The similarities with dishabituation are obvious. However, this type of neurone has not been reported in the CN, IC or MG, and until it is, the theory remains purely hypothetical.

Explanations based on synaptic inhibition have been specifically rejected by Thompson and Spencer (1966) as being incapable of explaining habituation in their acute spinal cats. They have based this rejection on two pieces of evidence:

(1) That the time course of pre- and post-synaptic inhibition is less than 500 milliseconds.

(2) That spontaneous recovery from habituation in their experiments was often of the order of 100 minutes after about 20 minutes stimulation at the rate of one per second.

The first point is not as crucial as they have thought, since Burke and Sefton (1966) have shown inhibitory processes which can last as long as ten seconds. The crucial nature of the second point for AEPH would depend on whether such processes can summate, and what the time course of recovery from AEPH turns out to be. This indicates that a study of spontaneous recovery could be important for a theoretical position based on synaptic inhibition.

4.6 Habituation as a Form of Learning

It has been proposed that habituation is a form of learning (Humphrey, 1933; Thorpe, 1950). Thorpe argues that habituation represents learning not to make a response, i.e., "negative learning". He says there is a "need for some form of learning which saves the animal from wasting its energies in responses to stimuli which experience shows to be harmless or of no significance. Habituation exactly meets this need and is well nigh universal" (Thorpe, 1950, p.390).

Thorpe could be confusing two mechanisms in his argument. It does seem that most organisms have a mechanism or mechanisms to prevent such wasting of energy. However, it does not necessarily follow that the mechanism is based on learning. The problem here is to distinguish a change due to learning from a change due to non-learning or performance factors. The problem of determining whether any change in behaviour is due to a learning process or to a performance or non-learning process is a persistent problem in the study of behaviour (Kimble, 1961). One of the more successful criteria for distinguishing between two such processes is based on the stability of the observed change in behaviour. Berlyne (1967, p.4) brings this point out when he says that "since learning is generally taken to imply a relatively permanent change in behaviour we have to have a interval of say, 24 hours intervening between training and testing" to establish the process as being based on learning.

This criterion raises immediate problems if one wishes to argue that all forms of habituation are based on learning. It must be admitted that some forms of BRH can occur with a stimulus interval of 24 hours (or a train and test interval of 24 hours) (Harris, 1943). This fact is compatible with a learning explanation. Furthermore, the evidence that repeated sessions of habituation can lead to increasingly faster habituation decrements also suggests a learning process (Harris, 1943). But in contrast to this evidence, most cases of BRH reveal that recovery from the change takes place in several hours (Thompson and Spencer, 1966). When this occurs, it is difficult to argue that the original change is due to learning since learning has somehow been abolished in a very short period of time.

The evidence for the effects of repeated sessions suggest that BRH might involve both a learning and an habituation mechanism. For example, the recovery data suggest that BRH decrements are not initially based on learning, but if repeated sessions lead to a faster growth of the decrement (which also recovers), then the factor controlling the rate of growth might be a learning one.

It can be seen immediately that a learning explanation is difficult to apply to the AEPH decrements observed in the experiments reported in chapter 3. Firstly, a decrement did not occur with train and test intervals as short as 1/20 sec. Secondly, there was considerable immediate recovery when the rate of stimulation was decreased from 1/1 sec to 1/10 sec (Experiment 5). These observations suggest that AEPH decrements are not initially due to learning.

4.7 Summary

It is apparent from the above analysis that only the theory that AEPH is produced by conditioning of the middle ear muscles, can be subjected to a direct test. It is proposed to test this theory by using a set of stimuli with random interstimulus intervals to induce AEPH. The other theories have only been tested indirectly. However, some of the studies, on which these theories are based, have either methodological flaws or inadequately reported parameters. Thus the main conclusion of this chapter is that many of the studies which were intended as tests of the various theories need to be repeated with more stringent controls. The next chapter sets out a number of experiments which endeavour to test these theoretical positions.

CHAPTER 5

5.0 Experiments Testing Theories of Auditory Evoked Potential Habituation

5.1 Experiment 6 - Conditioning of the Middle Ear Muscles

5.1.1 Introduction and Aim

It was pointed out in section 4.2 that there was a necessary condition for the theory that AEPH depends on conditioning of the middle ear muscles. It is stated that when regular repetitive stimuli are presented, the middle ear muscles learn to contract anticipatorily to the time interval as a CS. The necessary condition is that the intervals between stimuli must be fixed and regular, otherwise it would not be possible for the muscles to make an anticipatory response so that they would be able to influence auditory input.

Two stimulus conditions were used in this experiment. The mean rate of stimulus presentation was the same for the two conditions. However, for one condition the interstimulus interval was fixed, whereas for the other it varied randomly. Thus for the former condition, the stimuli were presented at regular intervals, whilst in the latter condition the stimuli arrived irregularly and unpredictably.

5.1.2 Subjects and Electrode Implantation

Using techniques already described in 2.4.4 and 3.2.2, bipolar electrodes were implanted in the CN, IC and MG. Four placements in each nucleus were selected for study.

5.1.3 Stimulus Parameters and Test Environment

The auditory stimuli were 20 msec clicks of 85 and 105 db re .0002 microbar, measured as described in 3.2.3. The regular stimulation consisted of clicks presented with a fixed interstimulus interval of either 1/sec or 1/10 sec. These were produced by triggering a Tektronix 161 pulse generator with a Tektronix 162 waveform generator. The irregular stimulation consisted of a set of click stimuli with random intervals between stimuli but with an overall mean rate of 1/sec or 1/10 sec. These random clicks were produced

by placing a radio-active source near a Geiger counter such that it discharged randomly at mean rates proportional to the distance between the counter and the source. The Geiger counter pulses triggered a waveform generator set to dead times of 280 msec and two sec for 1/sec and 1/10 sec mean rates respectively. The pulses produced were recorded on magnetic tape. During an experimental session, these recorded pulses were used to trigger the Tektronix 161 pulse generators. The random nature of the inter-pulse intervals was indicated by their exponential distribution when a histogram of frequency versus inter-pulse intervals was plotted (Fig. 5-1). The clicks were delivered through two loudspeakers set in the test box (2.4.2). Recording techniques were similar to those described in section 2.4.3. The test environment was similar to that described in 2.4.2.

5.1.4 Experimental Design

Each animal was tested under each condition of intensity, rate and random or regular presentation. A different random order of testing was used for each cat. The length of a test session was 20 minutes. At least seven days were allowed to elapse between consecutive sessions for any one cat.

The control stimuli were six pulses given at random over a five minute period before testing commenced. These pulses were given with no interstimulus interval less than 30 seconds.

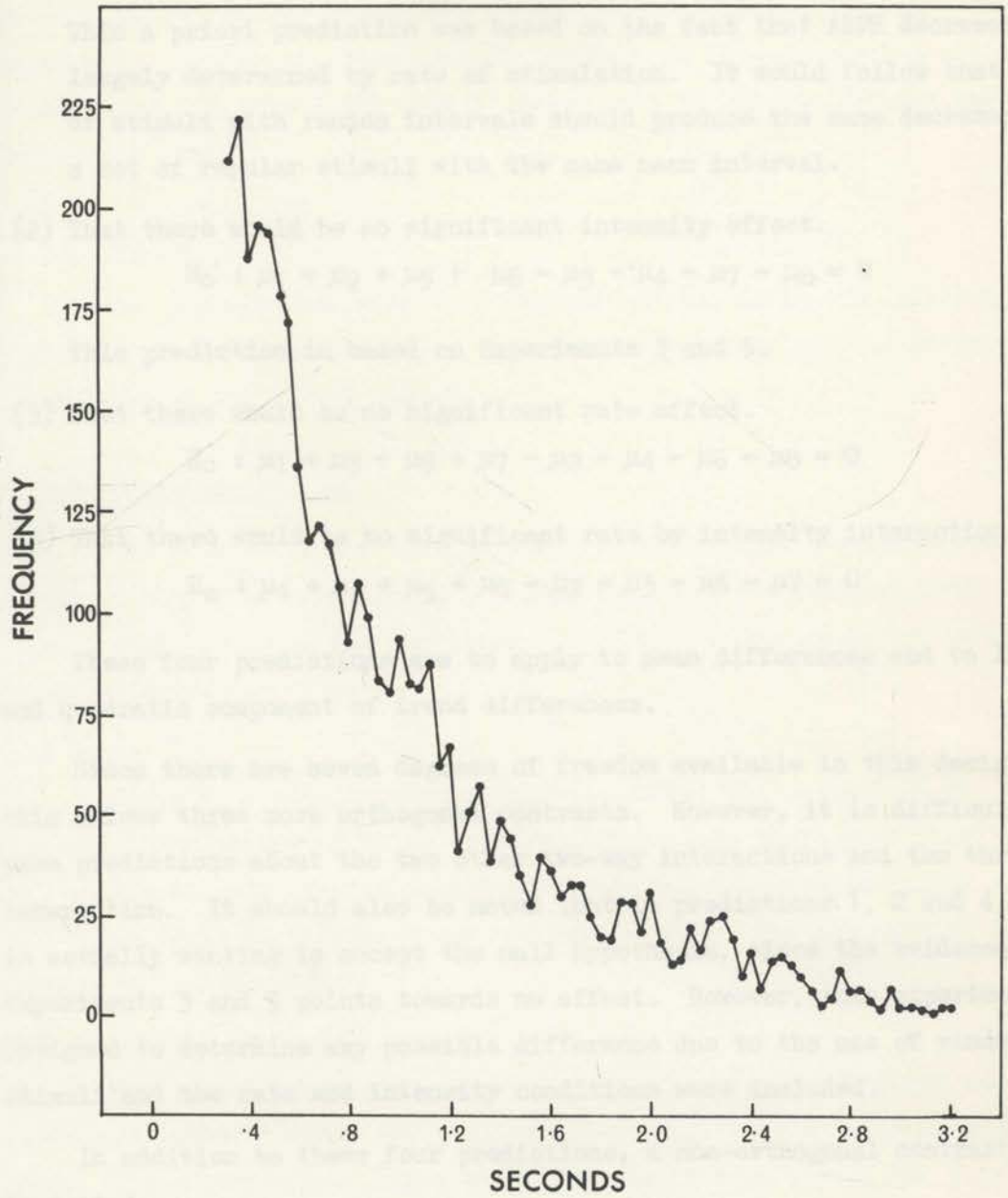
5.1.5 Data Reduction and Statistical Analysis

The data reduction was similar to that described in 3.3.5. The data were tested for robustness to lack of homogeneity of variance and analysed using the test of trend (3.2.6). The following figure sets out the means of the design of this experiment to assist the setting out of the planned contrasts.

		1/1	1/10
Random	85 db	μ_1	μ_2
	105 db	μ_3	μ_4
Regular	85 db	μ_5	μ_6
	105 db	μ_7	μ_8

Figure 5-1

Histogram of frequency versus inter-pulse intervals for a random set of intervals having a mean interval of one per second.


FREQUENCY
225
200
175
150
125
100
75
50
25
0
0
·4
·8
1·2
1·6
2·0
2·4
2·8
3·2
SECONDS

The following predictions were made for the test of trend. Each is an orthogonal contrast.

(1) That random stimulation would produce the same amount of habituation as regular stimulation.

$$H_0 : \mu_1 + \mu_2 + \mu_3 + \mu_4 - \mu_5 - \mu_6 - \mu_7 - \mu_8 = 0$$

This a priori prediction was based on the fact that AEPH decrements are largely determined by rate of stimulation. It would follow that a set of stimuli with random intervals should produce the same decrement as a set of regular stimuli with the same mean interval.

(2) That there would be no significant intensity effect.

$$H_0 : \mu_1 + \mu_2 + \mu_5 + \mu_6 - \mu_3 - \mu_4 - \mu_7 - \mu_8 = 0$$

This prediction is based on Experiments 3 and 5.

(3) That there would be no significant rate effect.

$$H_0 : \mu_1 + \mu_3 + \mu_5 + \mu_7 - \mu_2 - \mu_4 - \mu_6 - \mu_8 = 0$$

(4) That there would be no significant rate by intensity interaction.

$$H_0 : \mu_1 + \mu_4 + \mu_5 + \mu_8 - \mu_2 - \mu_3 - \mu_6 - \mu_7 = 0$$

These four predictions are to apply to mean differences and to linear and quadratic component of trend differences.

Since there are seven degrees of freedom available in this design, this allows three more orthogonal contrasts. However, it is difficult to make predictions about the two other two-way interactions and the three-way interaction. It should also be noted that in predictions 1, 2 and 4, one is actually wanting to accept the null hypothesis, since the evidence of Experiments 3 and 5 points towards no effect. However, this experiment was designed to determine any possible difference due to the use of random stimuli and the rate and intensity conditions were included.

In addition to these four predictions, a non-orthogonal contrast will be tested:

(5) That there would be no significant difference from zero slope for the combined random groups undergoing the faster rate of stimulation.

$$H_0 : \mu_1 + \mu_3 = 0$$

This is an important prediction, in the event that prediction (1) is not supported. That is, random stimulation might lead to less habituation than regular stimulation, but it might still give significant habituation. Thus the test of the conditioning theory could come about in two ways. If, however, prediction (1) is not supported, but prediction (5) is, then there would remain the problem of explaining the difference between regular and random stimulation. A type I and type II error rate of .05 was set for the experiment.

5.1.6 Results

The peak-to-peak amplitude studied was the deflection occurring within 250 msec of the stimulus. No attempt was made to analyse the different components of the evoked response.

5.1.6.1 Cochlear Nucleus

The results of the test of trend for percentage data summarised in Table 14 show that habituation has occurred since the overall trend is significant ($p < .001$) and the linear ($p < .05$) and the quadratic ($p < .01$) components are significant.

(a) Means

There is a significant difference between the means ($p < .01$). The specific predictions of 5.1.5 were supported since there is a significant effect due to rate of stimulation ($p < .01$), but both random versus regular and the effects of intensity are not significant. The interaction terms were also not significant.

(b) Components of trend

The between treatment trend was significant ($p < .001$) and the linear component between groups was significant ($p < .01$). When this is analysed in terms of the prediction, there is only a significant effect due to rate of stimulation ($p < .01$). Both the effects of random versus regular and the effects of intensity were non-significant. Groups 85/1/random and 105/1/random, when combined, were found to be significantly different from zero slope ($p < .001$). The quadratic components of trend fail to reach significance.

TABLE 14

SUMMARY OF ORTHOGONAL TREND ANALYSIS

Cochlear Nucleus - Experiment 6

SOURCE	DF	SS	MS	F
OVERALL TREND	10	.5136	.0514	4.42 ***
Linear	1	.2740	.2740	12.68 *
Quadratic	1	.0807	.0807	44.85 **
Residual	(8)	.1579	.0190	1.73
BETWEEN TREATMENT MEANS	7	.9446	.1349	2.72 *
Rate (R)	(1)	.5247	.5247	10.60 **
Intensity (I)	(1)	.0801	.0801	1.62
Random vs Regular (B)	(1)	.0421	.0421	.85
B x I	(1)	.0075	.0075	.15
B x R	(1)	.1026	.1026	2.07
R x I	(1)	.1869	.1869	3.78
B x R x I	(1)	.0007	.0007	.01
BETWEEN TREATMENT TRENDS	70	.8680	.0124	1.27
Linear	(7)	.2486	.0355	2.66 *
Rate (R)	(1)	.1377	.1377	10.31 **
Intensity (I)	(1)	.0293	.0293	2.20
Random vs Regular (B)	(1)	.0287	.0287	2.15
B x I	(1)	.0197	.0197	1.48
B x R	(1)	.0182	.0182	1.37
R x I	(1)	.0059	.0059	.44
B x R x I	(1)	.0089	.0089	.67
Quadratic	(7)	.0791	.0113	.63
Rate (R)	(1)	.0185	.0185	1.04
Intensity (I)	(1)	.0017	.0017	.10
Random vs Regular (B)	(1)	.0268	.0268	1.50
B x I	(1)	.0051	.0051	.29
B x R	(1)	.0173	.0173	.97
I x R	(1)	.0063	.0063	.36
B x R x I	(1)	.0033	.0033	.19
Residual	(56)	.5404	.0098	1.10
BETWEEN SUBJECT MEANS	3	.6771	.2257	
SUBJECT BY TREATMENT MEANS	21	1.0392	.4948	
BETWEEN SUBJECT TRENDS	30	.3482	.0116	
Linear	(3)	.0648	.0216	
Quadratic	(3)	.0054	.0018	
Residual	(24)	.2780	.0110	
SUBJECT BY TREATMENT TRENDS	210	2.0400	.0097	
Linear	(21)	.2803	.0133	
Quadratic	(21)	.3741	.0178	
Residual	(168)	1.3856	.0082	
TOTAL	351	6.4308		

Linear - Random 85/1/1 + 105/1/1 vs zero slope F = 26.98 (df 1,21) ***

Quadratic - Random 85/1/1 + 105/1/1 vs zero slope F = .94 (df 1,21)

The results of the statistical analysis show that habituation has occurred with random stimuli, and that in fact there is no significant difference between the degree of habituation produced by random and by regular stimulus presentation schedules. The only significant factor is rate of stimulus presentation. When the data were plotted as a function of time (Figs. 5-2 and 5-3), then the rate effect can be seen clearly, as can the lack of difference between random versus regular treatment conditions.

5.1.6.2 Inferior Colliculus

Significant overall habituation has also occurred in the IC (overall trend, $p < .001$; linear component, $p < .001$; quadratic component, $p < .001$) (Table 15).

(a) Means

There is a significant difference between treatment means ($p < .01$) and this is due entirely to the effects of rate of stimulation. There is no difference due to type of stimulus interval or intensity. None of the interaction terms is significant.

(b) Components of trend

The between treatment trend components is significant ($p < .05$). The overall linear trend between treatments is not significant but there is a difference due to rate of stimulation ($p < .05$), but no significant effects due to intensity or type of stimulus interval. The combination of the linear component rates for 85/1/random and 105/1/random differs significantly from zero slope. A similar result is obtained for the quadratic component of slope.

The results of the statistical analysis show that significant habituation has occurred to random stimuli and that there is no difference between either the effects due to random versus regular stimulation or the effects due to intensity. The significant variable is rate of stimulus presentation. When the data are plotted as a function of time, this rate effect can be clearly seen (Figs. 5-2 and 5-3).

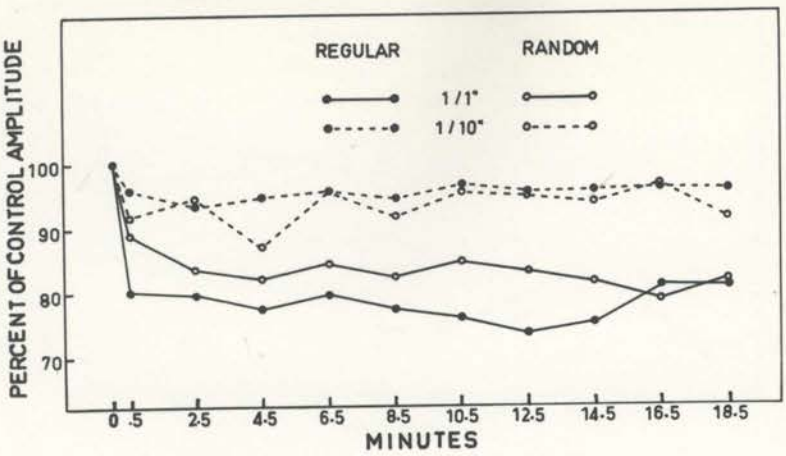
5.1.6.3 Medial Geniculate

Significant overall habituation has occurred at the MG (overall trend, $p < .001$; linear component, $p < .01$; quadratic component, $p < .05$) (Table 16).

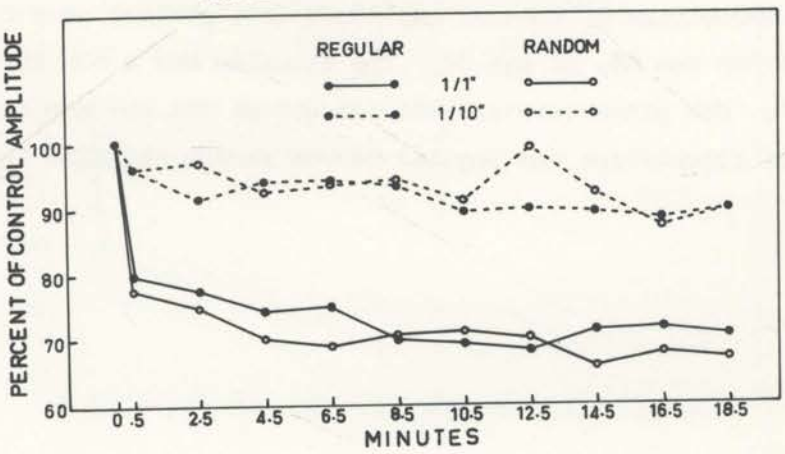
Figure 5-2

Percentage of control amplitude data plotted as a function of time for the CN, IC and MG. The stimulus was an 85 db, 20 msec "click". The parameters are one per second and one per ten second rates of stimulation and regular versus random stimulus intervals.

COCHLEAR NUCLEUS 85 db



INFERIOR COLLICULUS 85 db



MEDIAL GENICULATE 85 db

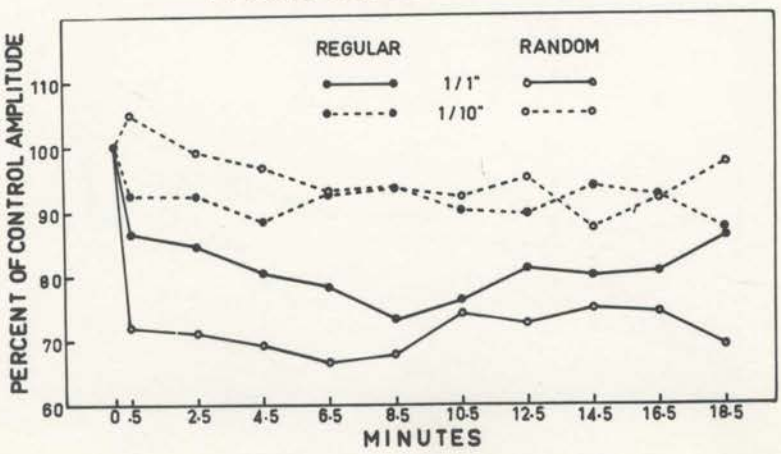
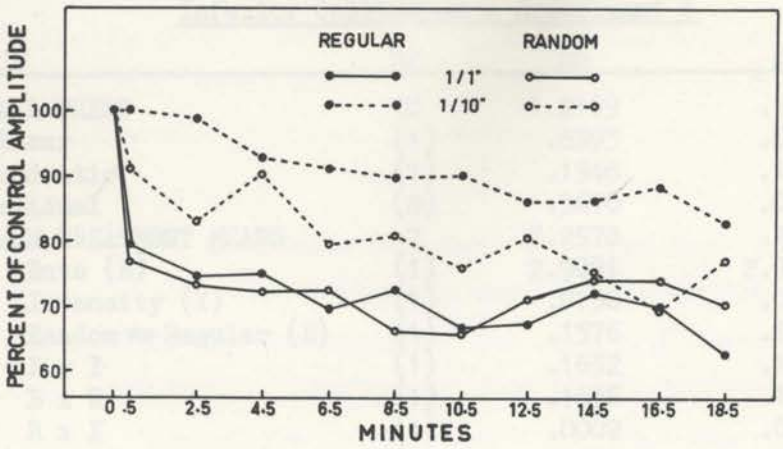


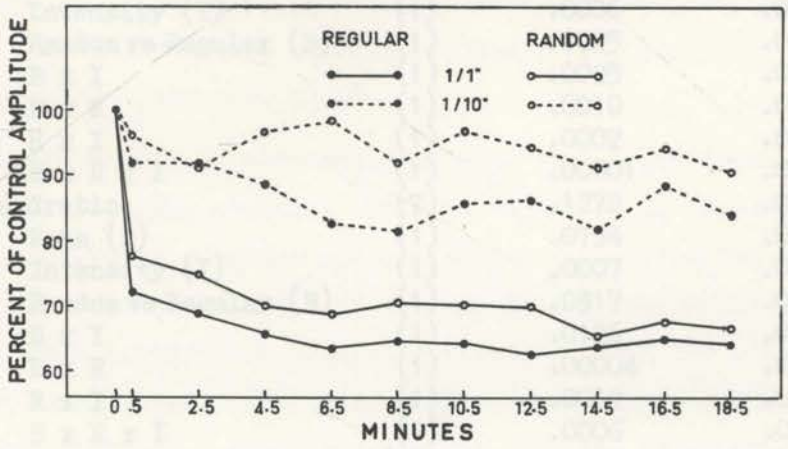
Figure 5-3

Percentage of control amplitude data plotted as a function of time for the CN, IC and MG. The stimulus was a 105 db, 20 msec "click". The parameters are one per second and one per ten second rates of stimulation and regular versus random stimulus intervals.

MEDIAL GENICULATE 105 db



INFERIOR COLLICULUS 105 db



COCHLEAR NUCLEUS 105 db

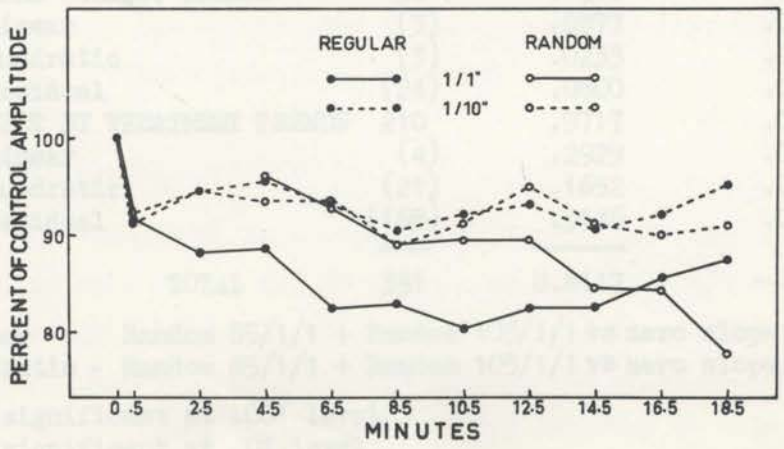


TABLE 15

SUMMARY OF ORTHOGONAL TREND ANALYSIS
Inferior Colliculus - Experiment 6

SOURCE	DF	SS	MS	F
OVERALL TREND	10	1.2169	.1217	19.11 ***
Linear	(1)	.6593	.6593	22.55 **
Quadratic	(1)	.1946	.1946	25.10 **
Residual	(8)	.3670	.0459	15.01 ***
BETWEEN TREATMENT MEANS	7	3.2578	.4654	4.46 **
Rate (R)	(1)	2.9091	2.9091	27.89 ***
Intensity (I)	(1)	.0150	.0150	.14
Random vs Regular (B)	(1)	.1376	.1376	1.32
B x I	(1)	.1632	.1632	1.57
B x R	(1)	.1636	.1636	.12
R x I	(1)	.0002	.0002	.01
B x R x I	(1)	.0259	.0259	.25
BETWEEN TREATMENT TRENDS	70	.4902	.4902	1.51 *
Linear	(7)	.0967	.0138	.99
Rate (R)	(1)	.0898	.0898	6.44 *
Intensity (I)	(1)	.0006	.0006	.04
Random vs Regular (B)	(1)	.0025	.0025	.18
B x I	(1)	.0025	.0025	.18
B x R	(1)	.0010	.0010	.07
R x I	(1)	.0002	.0002	.01
B x R x I	(1)	.00001	.00001	.001
Quadratic	(7)	.1272	.0182	2.31
Rate (R)	(1)	.0754	.0754	9.58 **
Intensity (I)	(1)	.0007	.0007	.09
Random vs Regular (B)	(1)	.0317	.0317	4.03
B x I	(1)	.0126	.0126	1.61
B x R	(1)	.00004	.00004	.0005
R x I	(1)	.0062	.0062	.79
B x R x I	(1)	.0006	.0006	.07
Residual	(56)	.2660	.0041	1.00
BETWEEN SUBJECT MEANS	3	.4944	.1648	
SUBJECT BY TREATMENT MEANS	21	2.1898	.1043	
BETWEEN SUBJECT TRENDS	30	.1910	.0064	
Linear	(3)	.0877	.0292	
Quadratic	(3)	.0233	.0078	
Residual	(24)	.0800	.0030	
SUBJECT BY TREATMENT TRENDS	210	.9717	.0046	
Linear	(4)	.2929	.0140	
Quadratic	(21)	.1652	.0079	
Residual	(168)	.5146	.0042	
TOTAL	351	8.8117		

Linear - Random 85/1/1 + Random 105/1/1 vs zero slope: F = 21.49, df (1,21)***
Quadratic - Random 85/1/1 + Random 105/1/1 vs zero slope: F = 9.530, df (1,21)**

*** significant at .001 level
** significant at .01 level
* significant at .05 level

(a) Means

There was a significant between treatment means effect ($p<.001$). There was a significant rate effect ($p<.001$) and a significant intensity effect ($p<.05$). There was no significant difference due to type of stimulus. The rate by intensity interaction was also significant ($p<.05$).

(b) Components of trend

The between treatments components of trend were not significant and the linear and the quadratic components were not significant. The predicted linear hypothesis was not supported for rate, but there was a significant intensity difference. There was no significant difference due to the type of stimulus. None of the interactions terms were significant. The combined random 85/1/1 and 105/1/1 data differed significantly from zero slope. The predicted quadratic component hypotheses were supported for a rate effect ($p<.05$), for intensity (non-significant) and for the type of stimulus (non-significant). The combined random 85/1/1 and 105/1/1 differed significantly from zero slope.

The results of this analysis show that significant habituation has occurred to random stimuli. This is supported by there being no significant difference between random and non-random conditions and by the significant difference from zero slope of the two faster random rates of stimulation. There was a significant intensity effect in both the means and the linear component of trend. This difference showed that the more intense stimulus (105 db) produced a greater decrement than the less intense stimulus (85 db). There was a significant rate effect for means and quadratic component of trend. When the data were plotted as a function of time (Figs. 5-2 and 5-3), these effects can be seen, and in particular the fact that a decrement occurred to random stimuli is evident.

5.1.7 Discussion

The results of this experiment clearly show that AEPH occurred with random stimuli at the CN, IC and the MG. This result refutes the theory that AEPH is due to the activation of the middle ear muscles by classical conditioning. According to this theory, the presentation of stimuli with a fixed interstimulus interval allows the interval to become a conditioned

TABLE 16

SUMMARY OF ORTHOGONAL TREND ANALYSIS
Medial Geniculate - Experiment 6

SOURCE	DF	SS	MS	F
OVERALL TREND	10	1.2322	.1232	13.30 ***
Linear	(1)	.6194	.6194	88.27 **
Quadratic	(1)	.3162	.3162	20.36 *
Residual	(8)	.2967	.0371	4.01 **
BETWEEN TREATMENT MEANS	7	2.4660	.3523	7.98
Rate (R)	(1)	1.7969	1.7969	40.72 ***
Intensity (I)	(1)	.2646	.2646	5.99 *
Random vs Regular (B)	(1)	.1110	.1110	2.51
B x I	(1)	.0065	.0065	.15
B x R	(1)	.0025	.0025	.06
R x I	(1)	.0157	.0157	.36
B x R x I	(1)	.2690	.2690	6.09 *
BETWEEN TREATMENT TRENDS	70	.5908	.0084	1.14
Linear	(7)	.1169	.0167	.87
Rate (R)	(1)	.00008	.00008	.004
Intensity (I)	(1)	.0907	.0907	4.75 *
Random vs Regular (B)	(1)	.0002	.0002	.01
B x I	(1)	.0028	.0028	.14
B x R	(1)	.0148	.0148	.77
R x I	(1)	.0027	.0027	.14
B x R x I	(1)	.0056	.0056	.29
Quadratic	(7)	.0868	.0124	1.05
Rate (R)	(1)	.0685	.0685	5.59 *
Intensity (I)	(1)	.0001	.0001	.01
Random vs Regular (B)	(1)	.0055	.0055	.46
B x I	(1)	.0024	.0024	.20
B x R	(1)	.0050	.0050	.42
R x I	(1)	.0005	.0005	.04
B x R x I	(1)	.0075	.0075	.64
Residual	(56)	.3871	.0071	.77
BETWEEN SUBJECT MEANS	3	.6911	.2304	
SUBJECT BY TREATMENT MEANS	21	.9267	.0441	
BETWEEN SUBJECT TRENDS	30	.2780	.0093	
Linear	(3)	.0211	.0070	
Quadratic	(3)	.0466	.0155	
Residual	(24)	.2103	.0091	
SUBJECT BY TREATMENT TRENDS	210	1.5583	.0074	
Linear	(21)	.4007	.0191	
Quadratic	(21)	.2472	.0118	
Residual	(168)	.9104	.0092	
TOTAL	351	7.7431		

Linear - Random 85/1/1 + 105/1/1 vs zero slope: F = 6.24 df (1,21) *
Quadratic - Random 85/1/1 + 105/1/1 vs zero slope: F = 14.34 df (1,21) **

*** significant at .001 level
** significant at .01 level
* significant at .05 level

stimulus for the precise activation of the middle ear muscles. This activation must be precisely timed so as to allow for the latency of middle ear muscle contractions. Consequently, the occurrence of habituation when the length of interstimulus interval varies randomly rules out the possibility of the interval acting as such a conditioned stimulus.

Once again, as in Experiments 3 and 5, there was a significant rate effect at each nucleus, However, there was a significant intensity effect at the MG. This was due to the more intense stimuli producing more habituation than the less intense stimuli. This result is in conflict with the results of Experiments 3 and 5, but it is similar to the results of Experiment 2. It suggests that the tests used in these experiments were not sufficiently sensitive to detect the small intensity effect. This result is in the opposite direction to that expected if AEPH had similar characteristics to that of BRH.

5.2 Experiment 7 - The Effects of Barbiturates on AEPH - I

5.2.1 Aim and Rationale

The aim of this experiment was to determine whether barbiturates abolished AEPH once it had been established. It was pointed out in section 4.3 that Hernández-Peón et al (1957) had argued that AEPH was produced by inhibition arising in the reticular formation. This position was largely based on the fact that surgical doses of barbiturate abolished AEPH at the CN. Two things can be said about this work. Firstly, many of the relevant stimulus parameters are not reported. Secondly, the marked amplitude decrements have not been observed in experiments reported in chapter 3. It was decided to examine the effects of barbiturate on AEPH decrements observed at the CN, IC and MG.

It has already been pointed out that there have been reports that barbiturates increase the relative refractory period of sensory systems (Chang, 1959). On the basis of this evidence, it would be expected that barbiturates would increase AEPH when fairly rapid rates of stimulation were used. A finding such as this would pose difficulties for the theory of afferent neuronal inhibition.

5.2.2 Subjects and Electrode Implantation

Bipolar electrodes were implanted in the CN, IC and MG of ten cats. Recordings were taken from six MG placements, four IC, and five CN. Some of the animals had already been used in Experiment 6. The implantation techniques were the same as those described in 2.4.4 and 3.2.2.

5.2.3 Stimulus Parameters, Test Environment, Design and Recording Techniques

The auditory stimuli were 20 msec clicks of 85 db re .0002 microbar. The stimuli were generated as described in section 5.1.3, so that the interstimulus interval varied randomly. The set of random intervals had a mean value of one per second. The test environment was similar to that described in section 2.4.2,with the clicks being delivered through the two loudspeakers. Each animal was stimulated for 20 minutes and then given an intraperitoneal injection of barbiturate (45 mg/Kg - Nembutal, Abbot).

When there were no signs of any reflex, the animal was placed in the same position that he had occupied before injection. Auditory stimulation was continued throughout. The animal was injected inside the test box. After all reflexes were abolished, each animal was stimulated for a further half hour. Thus the post-injection period was defined as beginning after all reflexes were abolished. Recording techniques were similar to those described in section 2.4.3.

5.2.4 Data Reduction and Statistical Analysis

The evoked potentials recorded on the Offner-Dymograph were measured in a way similar to that described in section 3.3.5. Two separate statistical analyses were carried out on the data as follows:

(1) The data for the pre-injection period were analysed with the test of trend after establishing that transformation of the data did not change the results of the analysis (3.2.6). This analysis was carried out to make sure that significant AEPH had occurred before the injection.

(2) The last 14 minutes of the pre-injection record and the first 14 minutes of the post-injection record were analysed with the test of trend (3.2.6). This was done to determine whether any change had taken place due to the injection. The last 14 minutes of the pre-injection period was chosen so that a reasonably asymptotic level could be compared with the effects of injection. A type I and type II error rate of .05 was set.

5.2.4.1 Histological Verification

All the placements used in this experiment were verified histologically using methods outlined in section 2.4.5.

5.2.5 Results

The peak-to-peak amplitude studied was the deflection occurring 250 msec after the impulse. No attempt was made to analyse the different components of the evoked response.

5.2.5.1 Cochlear Nucleus Results

The results of the tests of trend for percentage data are summarised in Table 17. The first half of the table contains the pre-injection analysis and the second half the comparison of pre- and post-injection conditions. It can be seen that significant overall habituation has occurred ($p < .001$) and the linear and quadratic components of trend are significant. These results indicate that significant AEPH has occurred before the injection (Fig.5-4).

The second half of Table 17 shows that there is no significant difference between the pre- and the post-injection records. This means that the injection of a surgical dose of barbiturate has not abolished AEPH. When the data are plotted as a function of time (Figure 5-4), it can be seen that there is a slight tendency for the post-injection record to be lower than the pre-injection level, but this difference is, of course, not statistically significant. Figure 5-5 shows evoked potential records which illustrate the pre-injection decrement and the subsequent post-injection changes.

5.2.5.2 Inferior Colliculus Results

Table 18 sets out the results of the test of trend for the pre-injection percentage data and the results of the test of trend for the comparison between pre- and post- conditions. Figure 5-4 shows the percentage data plotted as a function of time and there is a marked drop in amplitude in the post-injection record. The pre-injection analysis shows that significant AEPH has occurred prior to injection. The overall trend ($p < .001$), the linear ($p < .05$) and the quadratic components of trend ($p < .05$) are all significant.

The results of the comparison between the post- and pre-injection data show that the groups do not differ in components of trend, but that there is a significant difference between group means ($p < .01$). This difference is clearly shown in Figure 5-4. Thus the injection of a surgical dose of barbiturate has enhanced the amount of AEPH. Figure 5-5 shows evoked potential records for some of the placements. It can be seen that the drug has depressed the background noise activity as well as the amplitude of the evoked responses. This might indicate that the barbiturate has simply depressed all activity in the area.

TABLE 17

SUMMARY OF ORTHOGONAL TREND ANALYSIS

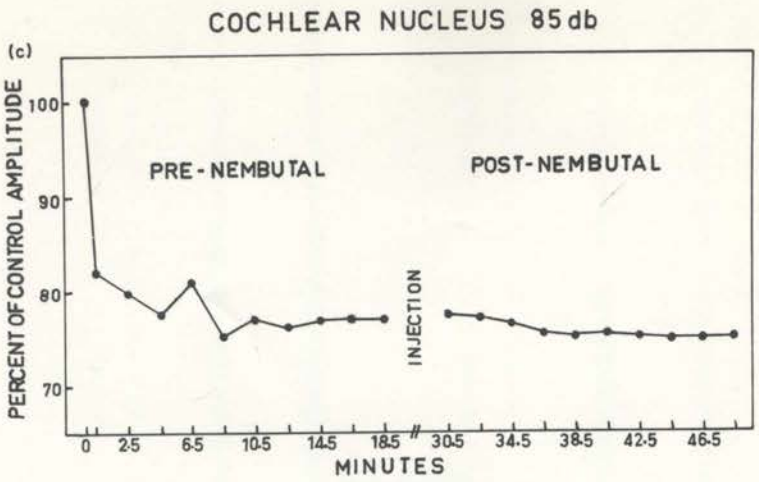
Cochlear Nucleus - Experiment 7

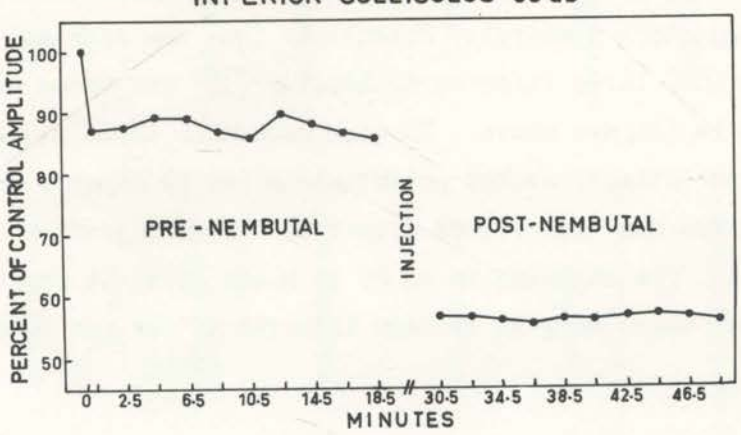
SOURCE	DF	SS	MS	F
(a) Pre-injection Data				
OVERALL TREND	10	.2464	.0246	14.36 ***
Linear	(1)	.0837	.0837	16.37 *
Quadratic	(1)	.0680	.0680	44.48 **
Residual	(8)	.0947	.0118	6.50 ***
BETWEEN SUBJECT TRENDS	40	.0686	.0017	
Linear	(4)	.0204	.0051	
Quadratic	(4)	.0061	.0015	
Residual	(32)	.0421	.0013	
BETWEEN SUBJECT MEANS	4	.1881	.0470	
TOTAL	54	.5031		
(b) Pre- and Post-injection Data				
OVERALL TREND	7	.0027	.0004	.64
Linear	(1)	.0011	.0011	4.36
Quadratic	(1)	.0009	.0009	.75
Residual	(5)	.0007	.0001	.30
BETWEEN TREATMENT MEANS	1	.0014	.0014	.05
BETWEEN TREATMENT TREND	7	.0025	.0004	.30
Linear	(1)	.0012	.0012	.35
Quadratic	(1)	.00001	.00001	.01
Residual	(8)	.0002	.0002	.29
BETWEEN SUBJECT MEANS	4	.5718	.1430	
SUBJECT BY TREATMENT MEANS	4	.1009	.0252	
BETWEEN SUBJECT TRENDS	28	.0169	.0006	
Linear	(4)	.0010	.0003	
Quadratic	(4)	.0047	.0012	
Residual	(20)	.0112	.0006	
SUBJECT BY TREATMENT TRENDS	28	.0332	.0012	
Linear	(4)	.0140	.0035	
Quadratic	(4)	.0043	.0011	
Residual	(20)	.0151	.0007	
TOTAL	79	.7925		

*** significant at .001 level
** significant at .01 level
* significant at .05 level

Figure 5-4

Three graphs showing the effect of barbiturate (Nembutal) on the percentage of control amplitude of responses recorded at the CN, IC and MG respectively. In each graph, pre- and post-injection data are shown. The stimulus was an 85 db click given with a mean random rate of one per second.


COCHLEAR NUCLEUS 85 db
(c)
PERCENT OF CONTROL AMPLITUDE
100
90
80
70
PRE-NEMBUTAL
POST-NEMBUTAL
INJECTION
0
2·5
6·5
10·5
14·5
18·5
30·5
34·5
38·5
42·5
46·5
MINUTES


INFERIOR COLLICULUS 85 db
PERCENT OF CONTROL AMPLITUDE
100
90
80
70
60
50
PRE-NEMBUTAL
INJECTION
POST-NEMBUTAL
0
2·5
6·5
10·5
14·5
18·5
30·5
34·5
38·5
42·5
46·5
MINUTES

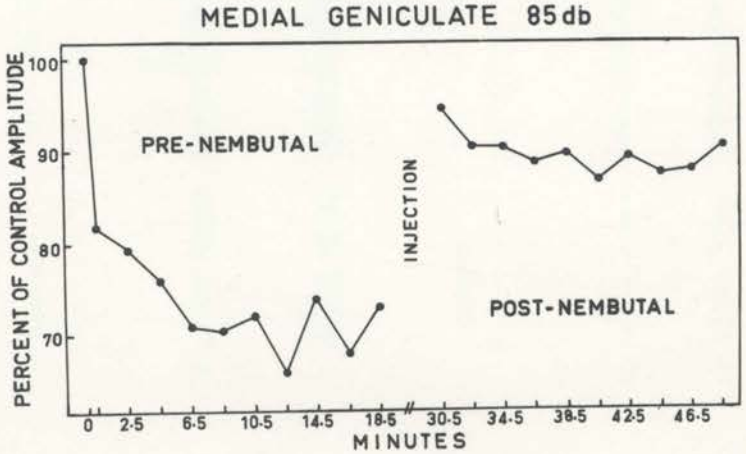
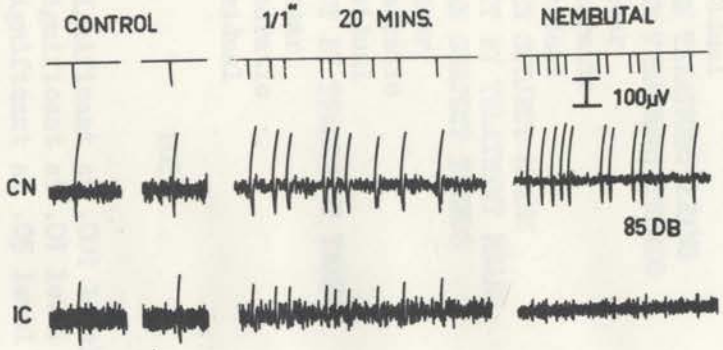
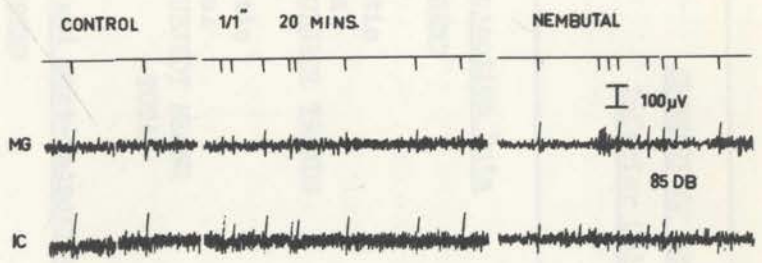
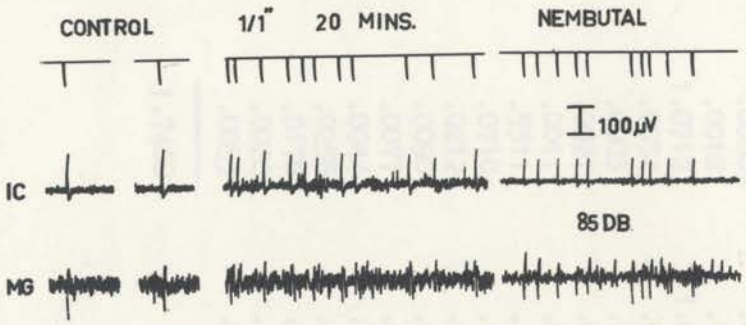

MEDIAL GENICULATE 85 db
PERCENT OF CONTROL AMPLITUDE
100
90
80
70
PRE-NEMBUTAL
INJECTION
POST-NEMBUTAL
0
2·5
6·5
10·5
14·5
18·5
30·5
34·5
38·5
42·5
46·5
MINUTES

Figure 5-5

This graph shows evoked potentials recorded before and after injecting Nembutal. Potentials from one cochlear nucleus (CN), three inferior colliculus (IC) and three medial geniculate (MG) are shown. In each record is shown control evoked potentials, evoked potentials after 20 minutes stimulation and then evoked potentials after injection of Nembutal. The stimulus is an 85 db click given at random intervals which have an average interval of one per second.


CONTROL
1/1" 20 MINS.
NEMBUTAL
100µV
CN
85 DB
IC


CONTROL
1/1" 20 MINS.
NEMBUTAL
100µV
MG
85 DB
IC


CONTROL
1/1" 20 MINS.
NEMBUTAL
100µV
IC
85 DB
MG

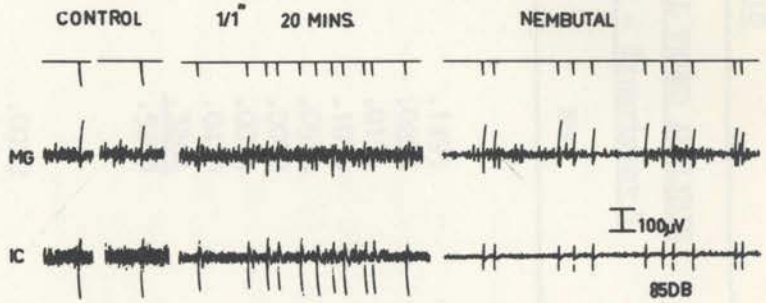

CONTROL
1/1" 20 MINS.
NEMBUTAL
MG
100µV
IC
85DB

TABLE 18

SUMMARY OF ORTHOGONAL TREND ANALYSIS

Inferior Colliculus - Experiment 7

SOURCE	DF	SS	MS	F
(a) Pre-injection Data				
OVERALL TREND	10	.1495	.0149	8.54 ***
Linear	(1)	.0288	.0288	11.05 *
Quadratic	(1)	.0151	.0151	32.58 *
Residual	(8)	.1056	.0112	10.20 ***
BETWEEN SUBJECT TRENDS	30	.0525	.0017	
Linear	(3)	.0078	.0026	
Quadratic	(3)	.0014	.0005	
Residual	(24)	.0433	.0011	
BETWEEN SUBJECT MEANS	3	.3264	.4088	
TOTAL	43	.5283		
(b) Pre- and Post-injection Data				
OVERALL TREND	7	.0022	.0003	.32
Linear	(1)	.0003	.0003	.10
Quadratic	(1)	.00005	.00005	.02
Residual	(5)	.0018	.0004	1.24
BETWEEN TREATMENT MEANS	1	1.0712	1.0712	45.23 **
BETWEEN TREATMENT TRENDS	7	.0034	.0005	.40
Linear	(1)	.0003	.0003	.05
Quadratic	(1)	.0021	.0021	2.23
Residual	(5)	.0011	.0002	1.50
BETWEEN SUBJECT MEANS	3	.5511	.1837	
SUBJECT BY TREATMENT MEANS	3	.0710	.0237	
BETWEEN SUBJECT TRENDS	21	.0212	.0010	
Linear	(3)	.0090	.0030	
Quadratic	(3)	.0077	.0026	
Residual	(15)	.0044	.0003	
SUBJECT BY TREATMENT TRENDS	21	.0258	.0012	
Linear	(3)	.0178	.0059	
Quadratic	(3)	.0028	.0009	
Residual	(15)	.0053	.0003	
TOTAL	63	1.7460		

*** significant at .001 level
** significant at .01 level
* significant at .05 level

5.2.5.3 Medial Geniculate Body Results

The results of the test of trend (Table 19) for the pre-injection data show that significant AEPH has occurred. The overall trend ($p < .001$) and the linear component of trend ($p < .01$) differ significantly from zero slope. When the percentage data are plotted as a function of time (Fig. 5-4), it can be seen that in contrast to the other two nuclei, the post-injection curve comes back towards the pre-habituation level. However, statistical analysis (Table 21) fails to find any significant difference between pre- and post-injection conditions. Figure 5-5 shows the evoked potential records for some of the cats. In this figure, it can be seen that there appears to be a large increase after injection, yet two other placements showed little change and another showed a small decrement in amplitude. It is probably this increase in variance which has prevented the test of trend from showing significant differences. If one looks closely at the evoked potential records that have increased in amplitude, it appears that there is a tendency for cyclic activity in the MG records after injection. These differences suggest that there could be placement differences in this nucleus.

5.2.6 Discussion

The results of the experiment appear to provide some evidence that is difficult for the theory of afferent neuronal inhibition to explain. This theory is based on the following arguments:

(1) Barbiturates are said to selectively block the reticular formation compared with other areas (Goodman and Gilman, 1960);

(2) Barbiturates abolish AEPH (Hernández-Peón et al, 1957; Al'tman, 1960).

It follows therefore that barbiturates are removing inhibition from the reticular formation. The results for the three nuclei are difficult for this theory. At the CN, there is no significant change after injection in contrast to the earlier studies. In some of the animals, there is a tendency for a slight increase in amplitude, but overall this is not statistically significant.

At the IC after injection, there is a marked depression of both the evoked potential amplitude and the background noise level. This could indicate that barbiturates increase AEPH, but the results are more in

TABLE 19

SUMMARY OF ORTHOGONAL TREND ANALYSIS

Medial Geniculate - Experiment 7

SOURCE	DF	SS	MS	F
(a) Pre-injection Data				
OVERALL TREND	10	.4490	.0449	7.28 **
Linear	(1)	.2114	.2114	20.22 **
Quadratic	(1)	.0925	.0925	6.27
Residual	(8)	.1451	.0181	4.50 ***
BETWEEN SUBJECT TRENDS	50	.3085	.0062	
Linear	(5)	.0523	.0105	
Quadratic	(5)	.0738	.0148	
Residual	(40)	.1824	.0045	
BETWEEN SUBJECT MEANS	5	1.1797	.2359	
TOTAL	65	1.9372		
(b) Pre- and Post-injection Data				
OVERALL TREND	7	.0306	.0044	1.36
Linear	(1)	.0136	.0136	3.57
Quadratic	(1)	.0006	.0006	.27
Residual	(5)	.0166	.0033	1.00
BETWEEN TREATMENT MEANS	1	.6902	.6902	2.91
BETWEEN TREATMENT TRENDS	7	.0202	.0029	1.47
Linear	(1)	.0017	.0017	.36
Quadratic	(1)	.0025	.0025	1.12
Residual	(5)	.0159	.0032	2.46
BETWEEN SUBJECT MEANS	5	1.1261	.2252	
SUBJECT BY TREATMENT MEANS	5	1.1837	.2367	
BETWEEN SUBJECT TRENDS	35	.1124	.0032	
Linear	(5)	.0190	.0038	
Quadratic	(8)	.0105	.0021	
Residual	(25)	.0830	.0033	
SUBJECT BY TREATMENT MEANS	35	.0684	.0020	
Linear	(5)	.0235	.0047	
Quadratic	(5)	.0113	.0023	
Residual	(25)	.0336	.0013	
TOTAL	95	3.2316		

*** significant at .001 level
** significant at .01 level
* significant at .05 level

keeping with other evidence that barbiturates tend to prolong the relative refractory period (Chang, 1959). (It should be pointed out that these studies did not involve the study of a chronic preparation with and without barbiturate. Instead a comparison was made between acute preparations and decerebrate animals.) In the present experiment there is such a large reduction of both the evoked potentials and the background noise, that it seems to indicate that there has been a marked depression in the ability of the nucleus to recover. It also indicates that the IC might be very sensitive to the action of barbiturates compared with the other two nuclei. It is also possible, of course, that an enhancement due to abolition of reticular formation inhibition is masked by a selective effect of barbiturates in this area. However, this proposition would be virtually untestable.

The results obtained at the MG are even more varied. There was not a statistically significant change in response amplitude, which in itself does not support the theory of afferent neuronal inhibition. However, a closer examination of the records of each of the six animals reveals marked variation after injection. Three placements increased in amplitude to a level which exceeded the control level. Two showed no change, and one a small decrement. In addition, the placements showing increases also had a tendency for cyclic activity to appear (Fig. 5-5). This cyclic activity was similar to that reported in the MG by Anderson et al (1967). It could suggest that a qualitative change has taken place in the waveform of the evoked potentials. This point will be examined in more detail in later sections. In general, the results of this experiment are difficult for an explanation based on reticular formation inhibition and they indicate that further experiments are needed on this problem.

5.3 Experiment 8 - The Effects of Barbiturates on AEPH - II

5.3.1 Rationale and Aim

Experiment 7 indicated that it is unlikely that inhibition generated in the reticular formation is responsible for evoked potential amplitude decrements observed at the CN and IC. However, the results observed at the MG showed a tendency to be more in keeping with the concept of reticular formation inhibition. Although the overall change produced by barbiturate was not statistically significant, some of the placements showed marked changes. In particular, there were indications that qualitative changes in waveform had been produced and it is difficult to interpret such changes in relation to the reticular formation as against solely amplitude changes.

The only theory of AEPH to be supported by the results of Experiment 7, was the theory of intrinsic inhibition. In this theory, it is postulated that each auditory stimulus evokes a field potential which terminates in a long-lasting (order of seconds) period of depressed excitability. This depression will dissipate, perhaps exponentially, but if another stimulus occurs during its presence, it will evoke a field potential of reduced amplitude. If this depression tends to be cumulative, then increasing the rate of stimulation will increase the amount of depression. Thus if barbiturates tend to depress the recovery cycle of areas such as the IC, then it would be expected that barbiturates would increase the amount of AEPH.

There is, however, another implication of this position with regard to the action of barbiturate. Although barbiturate might increase the amount of depression, it should still leave a relative difference between the effects of presenting stimuli with short and long intervals. With regard to the MG, it is possible that the qualitative changes produced by barbiturate are masking the effects of a long versus a short stimulus interval.

One way of testing these implications would be to habituate with a set of stimuli with random intervals and then inject barbiturate. Instead of averaging all the stimuli over a certain period, if the random intervals were categorised into very short intervals, short intervals and longer intervals, and each category were averaged, then this procedure would allow an assessment of the effect of the interval independently of the effects of

barbiturate. Or to use the terminology of earlier experiments, it would allow separation out of rate effects from barbiturate effects.

5.3.2 Subjects and Stimulus Parameters

Three placements were chosen at random from the CN and IC placements used in Experiment 7. The three MG placements showing increases were selected for this experiment. The auditory stimuli were again 20 msec "clicks" of 85 db re .0002 dynes/cm^2. The stimuli were generated as described in section 5.1.3, so that there were random intervals between stimuli with a mean value of one per second. The test environment was similar to that described in section 2.4.2. Each animal was stimulated for 20 minutes and then given a intraperitoneal injection of barbiturate (45 mg/Kg - Nembutal, Abbott). Similar procedures to those described in section 5.2.3 were then employed.

5.3.3 Data Reduction and Statistical Analyses

Three interstimulus interval categories were set up:

(1) all intervals between 280 - 500 msec
(2) all intervals between 500 - 1,000 msec
(3) all intervals longer than 1,000 msec.

(It should be pointed out that since there was a dead time of 280 msec set in the Geiger counter (section 5.1.3), there can be no interval shorter than 280 msec.) To determine the category into which any particular interval fell, the following procedure was adopted. The distance between each stimulus event mark was determined from the output of the pen recorder. Knowing the paper speed, it was a simple matter to convert to milliseconds. Thus each evoked potential was assigned to an interval category. As in section 3.3.5, the evoked potentials in the first minute and every alternate minute were measured. The average amplitude of each category within each of these minutes was obtained.

Two separate statistical analyses were carried out on the data as follows:

(1) A test of trend on the pre-injection data,

(2) A test of trend of the pre- and post-injection data to determine the effects of barbiturates.

A type I and type II error rate of .05 was set. It was predicted that there would be a rate and an anaesthetic effect in both the pre- and post-injection data.

5.3.4 Results

The results were based on the peak-to-peak amplitude occurring 250 msec after the stimulus. No attempt was made to analyse the components of the evoked response.

5.3.4.1 Cochlear Nucleus Results

(a) Pre-injection Results

Table 20 summarises the pre-injection analysis. Significant overall AEPH has occurred (overall trend, $p < .001$; linear component, $p < .001$; and quadratic component, $p < .05$). There was no significant difference between treatment means, but there was a significant difference between treatment trends ($p < .001$), and this was due to the linear component of trend ($p < .001$). This analysis indicates that there is a significant difference between the interval categories.

(b) Pre- and Post-injection Results

Table 21 summarises the pre- and post-injection analysis. There is a significant difference between treatment means ($p < .001$) and between treatment trends ($p < .01$). The difference between means is due to rate ($p < .001$) and to barbiturate versus non-barbiturate ($p < .001$). There is a significant interaction between rate and anaesthetic condition ($p < .001$).

The linear component of between treatment trend was significant ($p < .01$), but there was no significant difference due to the effects of rate and anaesthetic. There was a significant interaction ($p < .01$). There was a significant quadratic component of trend ($p < .01$) in which there was a singificant effect due to rate of stimulation, but no difference due to anaesthetic. There was a significant interaction ($p < .001$).

TABLE 20

SUMMARY OF ORTHOGONAL TREND ANALYSIS

Cochlear Nucleus - Experiment 8
Pre-Injection Data

SOURCE	DF	SS	MS	F
OVERALL TREND	10	.1251	.0125	6.40 ***
Linear	(1)	.0531	.0531	1848.44 ***
Quadratic	(1)	.0378	.0378	79.01 *
Residual	(8)	.0344	.0043	1.87
BETWEEN TREATMENT MEANS	2	.0180	.0090	1.40
BETWEEN TREATMENT TRENDS	2	.1285	.0064	5.37 ***
Linear	(2)	.0522	.0261	84.76 ***
Quadratic	(2)	.0061	.0031	2.67
Residual	(16)	.0702	.0044	3.38 **
BETWEEN SUBJECT MEANS	2	.0814	.0407	
SUBJECT BY TREATMENT MEANS	4	.0258	.0064	
BETWEEN SUBJECT TRENDS	20	.0391	.0020	
Linear	(2)	.0001	.00003	
Quadratic	(2)	.0009	.00005	
Residual	(16)	.0381	.0023	
SUBJECT BY TREATMENT TRENDS	40	.0479	.0012	
Linear	(4)	.0012	.0003	
Quadratic	(4)	.0046	.0011	
Residual	(32)	.0420	.0013	
TOTAL	98	.4658		

*** significant at .001 level
** significant at .01 level
* significant at .05 level

TABLE 21

SUMMARY OF ORTHOGONAL TREND ANALYSIS
Cochlear Nucleus - Experiment 8
Pre- and Post- Injection Data

SOURCE	DF	SS	MS	F	
OVERALL TREND	7	.0091	.0013	.93	
Linear	(1)	.0014	.0014	4.77	
Quadratic	(1)	.0014	.0014	14.18	
Residual	(5)	.0064	.0012	.06	
BETWEEN TREATMENT MEANS	5	1.9474	.3895	114.31	***
Rate (R)	(2)	.9107	.4554	133.64	***
Anaesthetic (A)	(1)	.3620	.3620	106.24	***
R x A	(2)	.6747	.3374	99.01	***
BETWEEN TREATMENT TRENDS	35	.0717	.0020	2.34	**
Linear	(5)	.0234	.0247	5.20	**
Rate (R)	(2)	.0028	.0014	1.58	
Anaesthetic (A)	(1)	.0002	.0002	.27	
R x A	(2)	.0203	.0102	11.30	**
Quadratic	(5)	.0250	.0050	8.16	**
Rate (R)	(2)	.0137	.0068	11.18	**
Anaesthetic (A)	(1)	.000007	.000007	.01	
R x A	(2)	.0113	.0056	9.22	**
Residual	(25)	.0235	.0009	1.00	
BETWEEN SUBJECT MEANS	2	.0509	.0255		
SUBJECT BY TREATMENT MEANS	10	.0341	.0034		
BETWEEN SUBJECT TRENDS	14	.0194	.0014		
Linear	(2)	.0006	.0003		
Quadratic	(4)	.0002	.0001		
Residual	(10)	.0188	.0018		
SUBJECT BY TREATMENT TRENDS	70	.0612	.0009		
Linear	(10)	.0090	.0009		
Quadratic	(10)	.0061	.0006		
Residual	(50)	.0462	.0009		
TOTAL	143	2.1939			

*** significant at .001 level

** significant at .01 level

* significant at .05 level

These results indicate that the injection of barbiturate depresses the activity in the CN and that there is an effect due to rate present in both the pre- and post-injection data. When the percentage data are plotted as a function of time, these effects can be clearly seen (Fig. 5-6). The significant interaction indicates that the effect of rate differs for the non-anaesthetic and the anaesthetic state. It appears that the rate effect is diminished under barbiturate.

5.3.4.2 Inferior Colliculus Results

(a) Pre-Injection Results

Table 22 summarises the pre-injection test of trend. The analysis shows that overall AEPH has occurred (overall trend, $p < .001$; linear component, $p < .001$; quadratic component, $p < .001$). There was a significant difference between treatment means ($p < .05$). The lack of a significant between treatment trend effect indicates that the groups have similar growth curves (Fig. 5-6).

(b) Pre and Post-injection Results

Table 23 summarises the test of trend for the pre- and post-injection data. There is a significant difference between group means ($p < .001$). This is due to a significant rate effect ($p < .01$) and a significant anaesthetic effect ($p < .001$). There is also a significant interaction ($p < .01$). A similar pattern occurs in the between groups linear component of trend. There were no significant differences in the quadratic component of trend.

These results show that the injection of barbiturate has depressed the activity in the inferior colliculus. There is a significant rate effect, but the significant interactions indicates that there is a difference in the effects of rate between the anaesthetic and non-anaesthetic data. Figure 5-6 shows the percentage amplitude data plotted as a function of time.

5.3.4.3 Medial Geniculate Results

(a) Pre-injection results

Table 24 summarises the test of trend for the pre-injection data. Overall AEPH has occurred (overall trend, $p < .01$; linear component, $p < .01$).

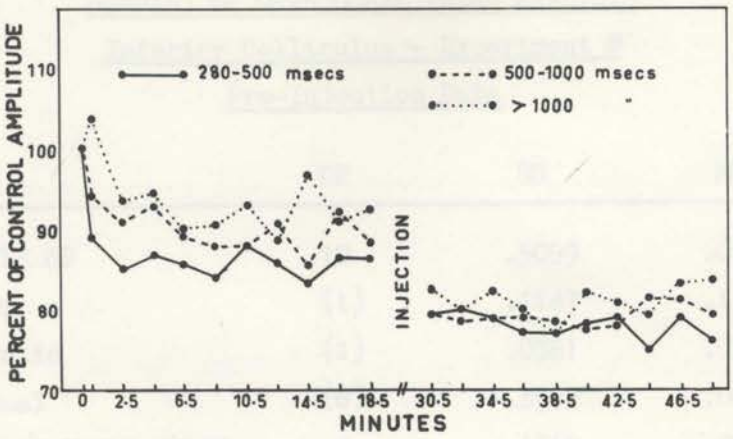
Figure 5-6

(a) The top graph shows percentage of control data plotted as a function of time for evoked potentials recorded in the CN. The curves up to the 18.5 minute mark are obtained before injecting Nembutal and the curves after this are obtained under Nembutal. The stimulus is an 85 db click given at random intervals which average one per second. The parameters are intervals between 280 - 500 msecs, 500 - 1000 msecs and intervals greater then 1000 msecs.

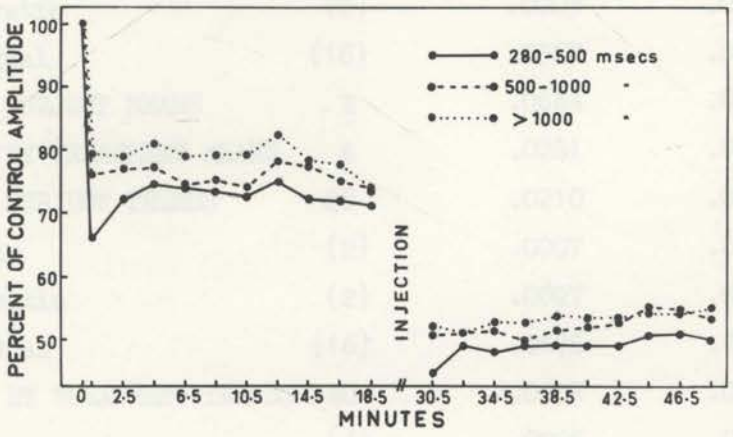
(b) The middle graph shows similar plots for the IC.

(c) The bottom graph shows similar plots for the MG.

COCHLEAR NUCLEUS 85db



INFERIOR COLLICULUS 85db



MEDIAL GENICULATE 85db

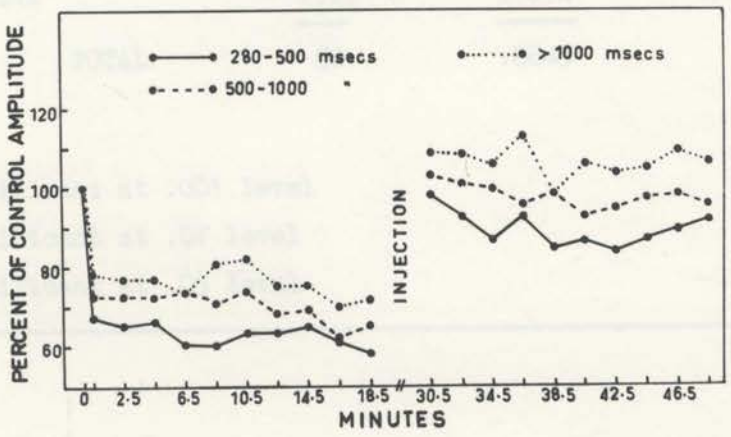


TABLE 22

SUMMARY OF ORTHOGONAL TREND ANALYSIS

Inferior Colliculus - Experiment 8

Pre-Injection Data

SOURCE	DF	SS	MS	F
OVERALL TREND	10	.5099	.0510	48.62 ***
Linear	(1)	.1147	.1147	342.24 ***
Quadratic	(1)	.0381	.0381	27.83 **
Residual	(8)	.3572	.0449	40.80 ***
BETWEEN TREATMENT MEANS	2	.1562	.0781	11.11 *
BETWEEN TREATMENT TRENDS	20	.0405	.0020	1.27
Linear	(2)	.0036	.0018	2.73
Quadratic	(2)	.0003	.0001	.26
Residual	(16)	.0367	.0023	1.64
BETWEEN SUBJECT MEANS	2	.0644	.0322	
SUBJECT BY TREATMENT MEANS	4	.0281	.0070	
BETWEEN SUBJECT TRENDS	20	.0210	.0010	
Linear	(2)	.0007	.0003	
Quadratic	(2)	.0027	.0014	
Residual	(16)	.0176	.0011	
SUBJECT BY TREATMENT TRENDS	40	.0637	.0016	
Linear	(4)	.0026	.0007	
Quadratic	(4)	.0020	.0005	
Residual	(32)	.0590	.0014	
TOTAL	98	.8843		

*** significant at .001 level

** significant at .01 level

* significant at .05 level

TABLE 23

SUMMARY OF ORTHOGONAL TREND ANALYSIS

Inferior Colliculus - Experiment 8
Pre- and Post-Injection Data

SOURCE	DF	SS	MS	F	
OVERALL TREND	7	.0060	.0009	1.94	
Linear	(1)	.00002	.00002	.11	
Quadratic	(1)	.0002	.0002	.38	
Residual	(5)	.0057	.0011	11.00	***
BETWEEN TREATMENT MEANS	5	2.6976	.5395	51.19	***
Rate (R)	(2)	.2456	.1228	11.65	**
Anaesthetic (A)	(1)	2.1634	2.1634	205.26	***
R x A	(2)	.2887	.1443	13.69	**
BETWEEN TREATMENT TRENDS	35	.0986	.0028	4.42	***
Linear	(5)	.0541	.0108	18.85	***
Rate (R)	(2)	.0232	.0116	20.21	***
Anaesthetic (A)	(1)	.0121	.0121	21.14	***
R x A	(2)	.0188	.0094	16.34	***
Quadratic	(5)	.0233	.0045	2.31	
Rate (R)	(2)	.0065	.0032	1.68	
Anaesthetic (A)	(1)	.0005	.0005	2.36	
R x A	(2)	.0113	.0056	2.92	
Residual	(25)	.0222	.0008	2.00	*
BETWEEN SUBJECT MEANS	2	.0678	.0339		
SUBJECT BY TREATMENT MEANS	10	.1054	.0105		
BETWEEN SUBJECT TRENDS	14	.0062	.0004		
Linear	(2)	.0033	.0016		
Quadratic	(2)	.0012	.0006		
Residual	(10)	.0017	.0001		
SUBJECT BY TREATMENT TRENDS	70	.0446	.0006		
Linear	(10)	.0057	.0006		
Quadratic	(10)	.0193	.0019		
Residual	(50)	.0195	.0004		
TOTAL	143	3.0262			

*** significant at .001 level

** significant at .01 level

* significant at .05 level

TABLE 24

SUMMARY OF ORTHOGONAL TREND ANALYSIS

Medial Geniculate - Experiment 8

Pre-Injection Data

SOURCE	DF	SS	MS	F
OVERALL TREND	10	.8281	.0828	42.19 **
Linear	(1)	.3419	.3419	131.01 **
Quadratic	(1)	.0407	.0407	9.66
Residual	(8)	.4455	.0556	37.06 ***
BETWEEN TREATMENT MEANS	2	2.0543	1.0272	110.25 **
BETWEEN TREATMENT TRENDS	20	.3942	.0197	13.72 **
Linear	(2)	.0303	.0151	8.42 *
Quadratic	(2)	.1371	.0686	21.52 **
Residual	(16)	.1673	.0104	9.45 ***
BETWEEN SUBJECT MEANS	2	.2041	.1020	
SUBJECT BY TREATMENT MEANS	4	.0373	.0093	
BETWEEN SUBJECT TRENDS	20	.0393	.0020	
Linear	(2)	.0052	.0026	
Quadratic	(2)	.0084	.0042	
Residual	(16)	.0257	.0015	
SUBJECT BY TREATMENT TRENDS	40	.0575	.0014	
Linear	(4)	.0072	.0018	
Quadratic	(4)	.0127	.0032	
Residual	(32)	.0376	.0011	
TOTAL	98	3.6146		

*** significant at .001 level

** significant at .01 level

* significant at .05 level

There is a significant difference between group means ($p < .001$) and a significant difference between group trends ($p < .001$), between group linear component ($p < .05$) and quadratic component ($p < .01$). These results show a significant rate effect in the pre-injection data (Fig. 5-6).

(b) Pre- and Post-injection Results

Table 25 summarises the test of trend for the pre- and post-injection data. There is a significant difference between group means ($p < .001$). This is due to a significant rate effect ($p < .01$) and a significant difference due to barbiturate ($p < .001$). There is a significant interaction ($p < .001$). There is a significant linear component of trend ($p < .01$) and this is due to a significant rate effect ($p < .05$) and a significant interaction. The significant quadratic component ($p < .01$) is due to a significant effect of barbiturate.

These results indicate that there has been a significant increase in amplitude due to injection of barbiturate. But despite this increase, there is still a difference due to rate of stimulation. The significant interactions indicate that the rate effect is different under barbiturate compared with no barbiturate. These effects are clearly seen in Figure 5-6.

5.3.5 Discussion

The results obtained at the CN and the IC are not in keeping with predictions from the theory of afferent neuronal inhibition. It is clear that surgical doses of barbiturate have failed to abolish AEPH in these nuclei. In contrast to the specific prediction from this theory, the effect of barbiturate was to increase the level of AEPH. This result confirms, in part, the results of the last experiment. In that experiment, there was not a significant depression of the CN response by barbiturate.

However, in the present experiment, it is the influence of stimulus interval or rate of stimulation that is important. It is apparent that there is a rate effect both with and without barbiturate. If AEPH is largely a function of rate of stimulation, as the experiments in chapter 3 have indicated, then it is apparent that barbiturates are not abolishing this effect. The data suggest, in fact, that barbiturate is depressing the recovery cycle in each nucleus, over and above the effects of rate. This

TABLE 25

SUMMARY OF ORTHOGONAL TREND ANALYSIS

Medial Geniculate - Experiment 8
Pre- and Post-Injection Data

SOURCE	DF	SS	MS	F	
OVERALL TREND	7	.1241	.0177	6.70	**
Linear	(1)	.0916	.0916	34.35	*
Quadratic	(1)	.0007	.0007	.45	
Residual	(5)	.0317	.0063	2.25	
BETWEEN TREATMENT MEANS	5	5.6807	1.1361	97.76	***
Rate (R)	(2)	.3178	.1589	13.67	**
Anaesthetic (A)	(1)	2.8589	2.8589	245.99	***
R x A	(2)	2.5040	1.2520	107.73	***
BETWEEN TREATMENT TRENDS	35	.2517	.0072	3.92	***
Linear	(5)	.0887	.0177	6.65	**
Rate (R)	(2)	.0277	.0139	5.20	*
Anaesthetic (A)	(1)	.00003	.00003	.01	
R x A	(2)	.0609	.0304	11.41	**
Quadratic	(5)	.0380	.0076	6.29	**
Rate (R)	(2)	.0024	.0012	1.01	
Anaesthetic (A)	(1)	.0340	.0340	28.12	***
R x A	(2)	.0016	.0008	.65	
Residual	25	.1251	.0050	2.63	**
BETWEEN SUBJECT MEANS	2	.5523	.2761		
SUBJECT BY TREATMENT MEANS	10	.1162	.0116		
BETWEEN SUBJECT TRENDS	14	.0370	.0026		
Linear	(2)	.0053	.0027		
Quadratic	(2)	.0031	.0016		
Residual	(10)	.0286	.0028		
SUBJECT BY TREATMENT TRENDS	70	.1284	.0018		
Linear	(10)	.0267	.0027		
Quadratic	(10)	.0121	.0012		
Residual	(50)	.0998	.0019		
TOTAL	143	6.8904			

*** significant at .001 level

** significant at .01 level

* significant at .05 level

result is more in keeping with the theory of intrinsic inhibition and with other observations about barbiturates (Chang, 1959). The significant interactions between rate and anaesthetic indicate that the differential effect of rate is less under anaesthetic.

The interpretation of the results obtained at the MG is more complex. There is an increase in evoked response amplitude after injection of barbiturate and this would appear to confirm predictions from the theory of afferent neuronal inhibition. However, two factors suggest that this interpretation might not be correct:

(1) These nuclei have once again shown signs of changes in their waveform after injection.

(2) The rate effect which is present before the injection is still present after injection, despite the large increase in response amplitude. When this observation is taken in conjunction with the first point, it suggests that barbiturates are producing amplitude increments and waveform changes and they are not releasing a system from decrements due to rate of stimulation. That is, the mechanism inducing the rate effect is still operating on the barbiturate modified potentials.

It could be argued that all these effects are produced by barbiturates acting on the reticular formation and that the changes appear to be more complex than the earlier workers have reported. This is a possible defence of the theory of afferent neuronal inhibition, but if accepted, it would suggest that systemic injections of barbiturates are not a convincing test of this theory.

Al'tman (1960) has also reported that barbiturates prevent the development of AEPH at the MG. Now, it is possible to argue, on the basis of the present experiment, that barbiturates could produce changes in the MG that would obscure any AEPH. Moreover, if barbiturates produced these marked changes in waveform without any prior habituation stimuli, then it would be difficult to compare these responses to those obtained without barbiturate. It would raise the problem of which response is habituating; is it the response under anaesthetic or the response without anaesthetic? The results of Experiment 8 also raise the possibility that both responses

would habituate. However, a test of this inference would need to compare habituated responses with control responses obtained under the same conditions, that is, with or without barbiturate. This type of experiment has not been previously reported and could be a crucial test of the role of barbiturate.

Finally, it must be pointed out that the MG placements studied in this experiment were selected because they showed amplitude increases under barbiturate. Thus the observation that the rate effect is still present under barbiturate, might not be a general finding. Further experiments with this nucleus are required to establish the generality of the finding.

5.4 Experiment 9 - The Effects of Barbiturates on AEPH - III

5.4.1 Rationale and Aim

The results of Experiments 7 and 8 have indicated that AEPH at the CN and IC is not produced by inhibition from the reticular formation, although the results obtained at the MG have been more in keeping with this hypothesis. However, two features of the geniculate results suggest that other factors may be involved. Firstly, it appeared that barbiturates were producing qualitative changes in the waveform of MG evoked potentials. This suggested that it might be difficult to interpret increases in evoked potential amplitude as a mere return to pre-habituation levels. Secondly, the results of Experiment 8 indicated that a rate effect was still apparent under barbiturate. This observation in conjunction with the qualitative change also suggests that barbiturates might be producing an effect independent of the one produced by rate of stimulation.

Some experimenters (Hernández-Peón et al, 1956; Al'tman, 1960) have reported that barbiturates prevent the development of AEPH. In these experiments, they did not obtain a pre-barbiturate record as a control. That is, they could not rule out the possibility that the action of barbiturates had so changed the evoked potential that any AEPH might be obscured.

In Experiments 7 and 8 and in the above experiments, relatively crude measures of evoked potentials were employed. It was thus decided to repeat this work at the MG using the Computer of Average Transients (C.A.T.), so that waveform changes would be easier to detect. Thus the aims of this experiment were:

(1) To study the effects of barbiturates on previously established AEPH at the MG using the C.A.T. to measure responses.

(2) To study the effects of barbiturates on the development of AEPH after administration of the drug, and to determine whether barbiturates produce changes apart from habituation effects.

5.4.2 Subjects, Stimulus Parameters and Design

Five MG placements already used in Experiment 5 were employed in this experiment. The auditory stimuli were 20 msec clicks of 105 db re .0002

dynes/cm^2 and were delivered by earphones on the animal's head.

The design of this experiment consisted of two parts:

(1) Five placements were stimulated for 30 minutes with clicks given regularly at the rate of one per second. Then a surgical dose of barbiturate (45 mg/Kg - Nembutal, Abbott) was administered by an intraperitoneal injection. Stimulation was continued and recording recommenced when there were no longer any signs of a reflex.

(2) Four placements were used in the second half of this experiment (one animal being rejected because of signs of middle ear disease). A pre-injection control was taken when each animal was stimulated with 50 clicks at one per ten seconds. After this they were injected with barbiturate, another control record was taken and then an habituation session commenced, when all signs of reflexes had been abolished. The habituation session consisted of 30 minutes stimulation with clicks given at the rate of one per second.

5.4.3 Recording Technique and Data Collection

All responses were analysed on line with the C.A.T., as described in section 3.5.4. Fifty responses were averaged. During the habituation session, the responses were always recorded so that the mid-point of the block of responses fell at a five-minute mark. The averaged responses were displayed on an X-Y plotter.

5.4.4 Data Reduction and Statistical Analyses

The printer output of the C.A.T. memory was punched onto IBM computer cards and analysed as described in section 3.5.7. The main variable measured was the peak-to-peak amplitude. No attempt was made to measure the area under the slow wave as there were obvious changes in the shape of the waveform. These changes made it impossible to compare areas of pre- and post-anaesthetic potentials.

The first half of the experiment was analysed with two separate tests of trend:

(1) A test of trend of the pre-injection data,

(2) A test of trend of the pre- and post-injection data.

The second half of the experiment was analysed by a test of trend on the post-anaesthetic habituation data and a t-test of the difference between the pre- and post-injection controls. Type I and type II error rates of .05 were set.

5.4.5 Results

The results of this experiment are shown in Figure 5-7. The first half of Table 26 summarises the pre-injection analysis of the first part of the experiment. The overall trend component is significant ($p<.05$). This indicates that significant AEPH has occurred before injection. The linear component of trend is also significant ($p<.05$). The second half of Table 26 summarises the comparison between the pre- and post-injection results. There is a significant difference between the two conditions, since the between treatment trends is significant ($p<.01$) and the between treatments linear component is also significant ($p<.05$). This analysis indicates that a significant change has taken place as a result of the injection of barbiturate. Figure 5-7 shows that there has been a marked increase in response amplitude after the injection. This result confirms the results of Experiments 7 and 8.

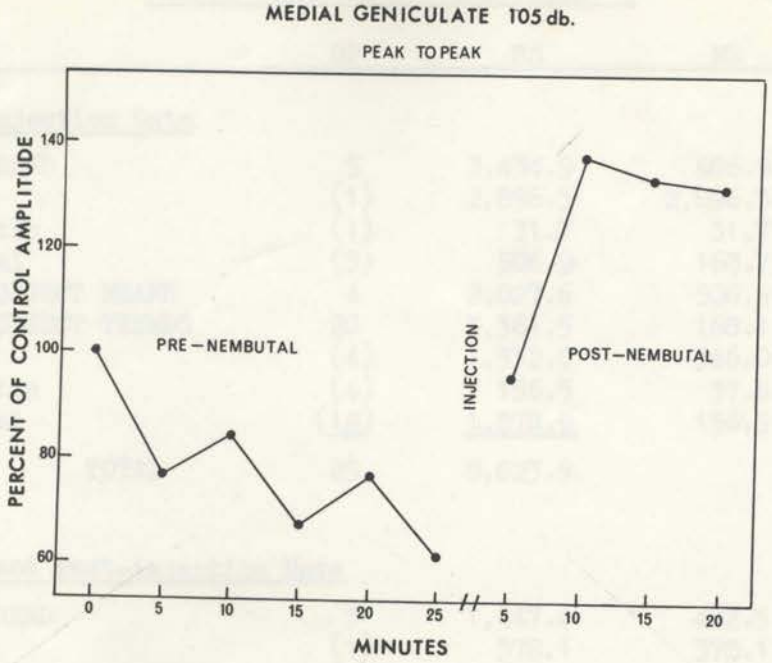
There have also been marked changes in the waveform of the MG potentials. In three animals, the waveform changes consisted of a cyclic activity with a period of approximately 100 msec. Figures 5-8, 5-9 and 5-10 show this effect quite clearly. This cyclic activity gradually diminished in amplitude with each cycle. This effect can be seen in Figure 5-11, when one of the MG responses was averaged with a time base of 1,000 msecs. The other two placements showed waveform changes, but there was no sign of the cyclic activity.

The data from the second half of the experiment are plotted as a function of time in the lower half of Figure 5-7. The test of trend of these data is summarised in Table 27. It can be seen that a small decrement in amplitude occurs over time when habituation is commenced after barbiturates are injected. This is confirmed by a significant linear trend component ($p<.05$). A t-test showed a significant difference between the pre- and post-injection controls ($t = 8.37$, $p<.01$, $df = 3$).

Figure 5-7

(Top Graph) Reduction in evoked potential amplitude with repetitive stimulation at 1/1 sec, followed by an increase in evoked potential amplitude after injection with barbiturate.

(Bottom Graph) Increase in evoked potential amplitude after injection with barbiturate without intervening repetitive stimulation, followed by a decrease in this new amplitude level with repetitive stimulation.


MEDIAL GENICULATE 105 db.
PEAK TO PEAK
PERCENT OF CONTROL AMPLITUDE
140
120
100
80
60
PRE—NEMBUTAL
INJECTION
POST—NEMBUTAL
0
5
10
15
20
25
5
10
15
20
MINUTES

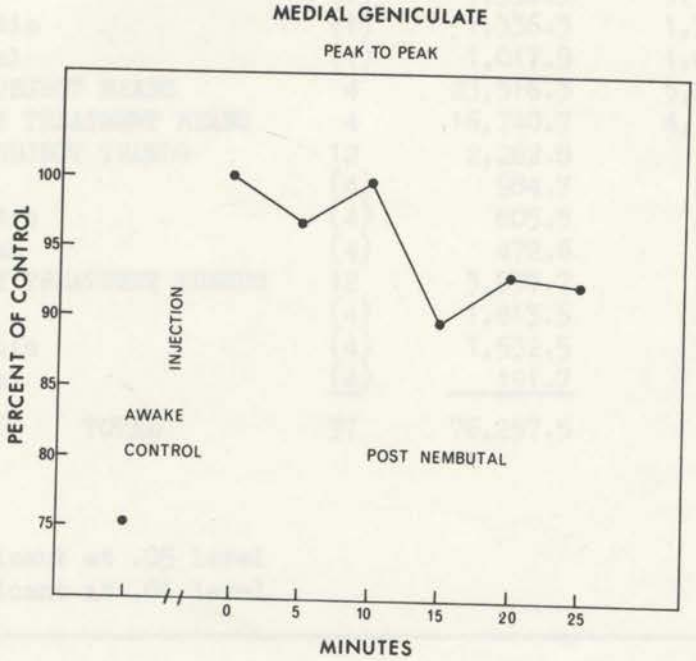

MEDIAL GENICULATE
PEAK TO PEAK
PERCENT OF CONTROL
100
95
90
85
80
75
INJECTION
AWAKE
CONTROL
POST NEMBUTAL
0
5
10
15
20
25
MINUTES

TABLE 26

SUMMARY OF ORTHOGONAL TREND ANALYSIS

Medial Geniculate - Experiment 9

SOURCE	DF	SS	MS	F
(a) Pre-injection Data				
OVERALL TREND	5	3,434.9	686.9	4.09 *
Linear	(1)	2,896.3	2,896.3	7.92 *
Quadratic	(1)	31.7	31.7	.84
Residual	(3)	506.9	168.7	1.08
BETWEEN SUBJECT MEANS	4	2,027.6	506.9	
BETWEEN SUBJECT TRENDS	20	3,361.5	168.1	
Linear	(4)	1,332.0	366.0	
Quadratic	(4)	156.5	37.6	
Residual	(12)	1,878.9	156.5	
TOTAL	29	8,823.9		
(b) Pre- and Post-injection Data				
OVERALL TREND	3	1,447.4	482.5	2.56
Linear	(1)	378.1	378.1	1.54
Quadratic	(1)	1,067.1	1,067.1	5.30
Residual	(1)	2.2	2.2	.01
BETWEEN TREATMENT MEANS	1	23,040.0	23,040.0	5.51
BETWEEN TREATMENT TRENDS	3	5,912.5	1,970.8	6.69 **
Linear	(1)	3,558.3	3,558.3	7.85 *
Quadratic	(1)	1,336.3	1,336.3	3,49
Residual	(1)	1,017.9	1,017.9	21.23 **
BETWEEN SUBJECT MEANS	4	23,316.3	5,829.1	
SUBJECT BY TREATMENT MEANS	4	16,740.7	4,185.2	
BETWEEN SUBJECT TRENDS	12	2,262.8	188.6	
Linear	(4)	984.7	246.2	
Quadratic	(4)	805.5	201.4	
Residual	(4)	472.6	118.1	
SUBJECT BY TREATMENT TRENDS	12	3,537.7	294.8	
Linear	(4)	1,813.5	453.4	
Quadratic	(4)	1,532.5	383.1	
Residual	(4)	191.7	47.9	
TOTAL	37	76,257.5		

* significant at .05 level
** significant at .01 level

Figure 5-8

This figure shows the effects of barbiturate on MG evoked potentials of one cat as follows:

A: X-Y plot of average of 50 responses to 105 db clicks given at rate of 1/10 sec (pre-habituation control),

B: X-Y plot of average of 50 responses to clicks after 30 minutes stimulation at 1/1 sec (habituated),

C: X-Y plot of average of 50 responses to clicks at 1/1 sec after injection of barbiturate,

D: Averaged response to 50 clicks at 1/10 sec (pre-habituated control),

E: Averaged response to 80 clicks at 1/10 sec after injection of barbiturate (pre-habituation barbiturate control),

F: Averaged response after 30 minutes stimulation at 1/1 sec.

The comparison of D and E shows that barbiturate produces changes apart from repetitive stimulation. Amplitude calibrations=50 μ volts; time=25 msecs.

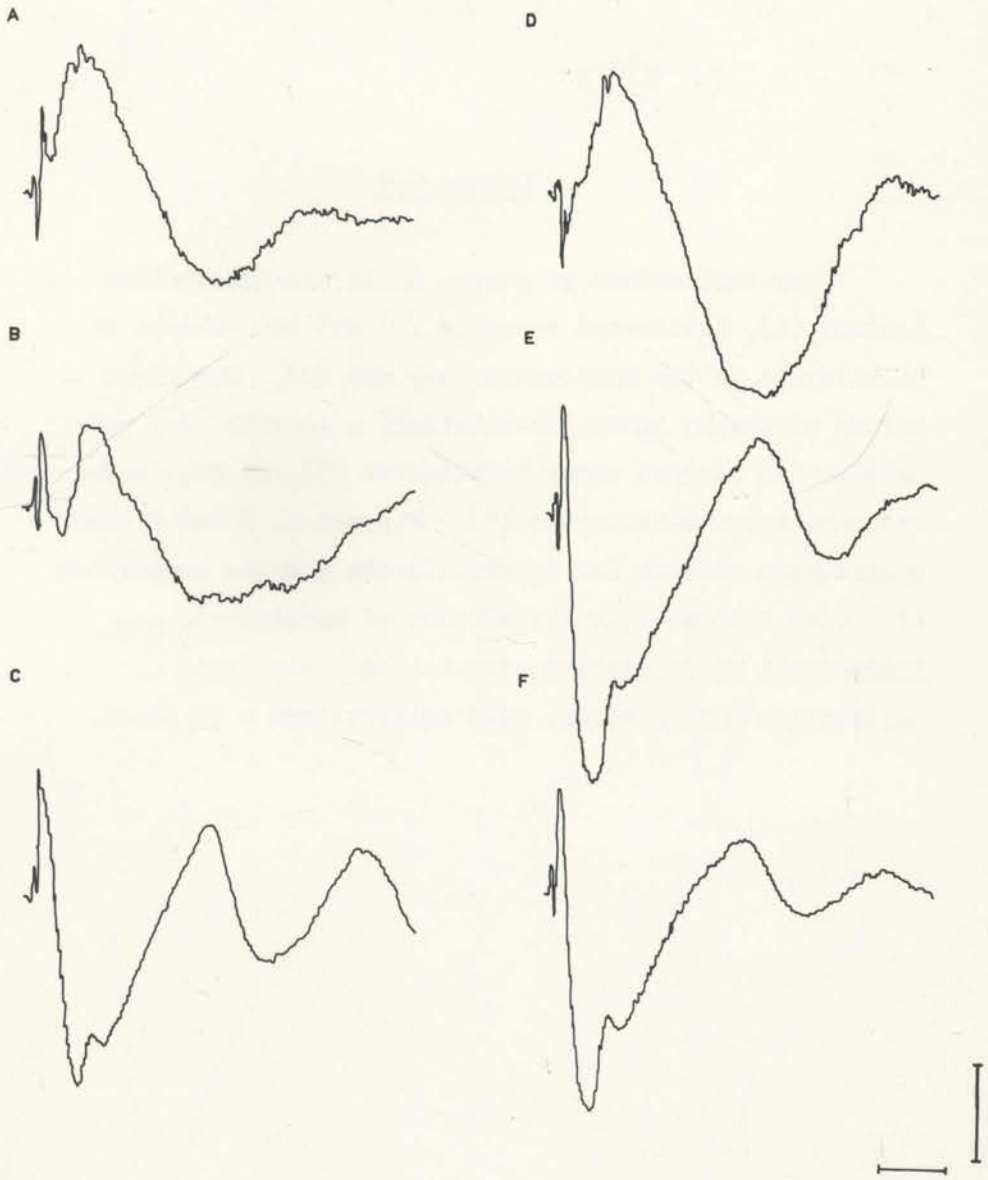

A
B
C
D
E
F

Figure 5-9

The left column of graphs shows pre-habituation control (A), habituated response (B) and the effects of barbiturate on the habituated response (C). The right column of graphs shows pre-habituation control (D), pre-habituation control under barbiturate (E) and habituated response under barbiturate (F). Figures C, E and F show qualitative changes due to barbiturate and the comparison of D with E shows that the effects of barbiturate are independent of repetitive stimulation. Amplitude calibration = 50 μ volts, time calibrations = 25 msecs.

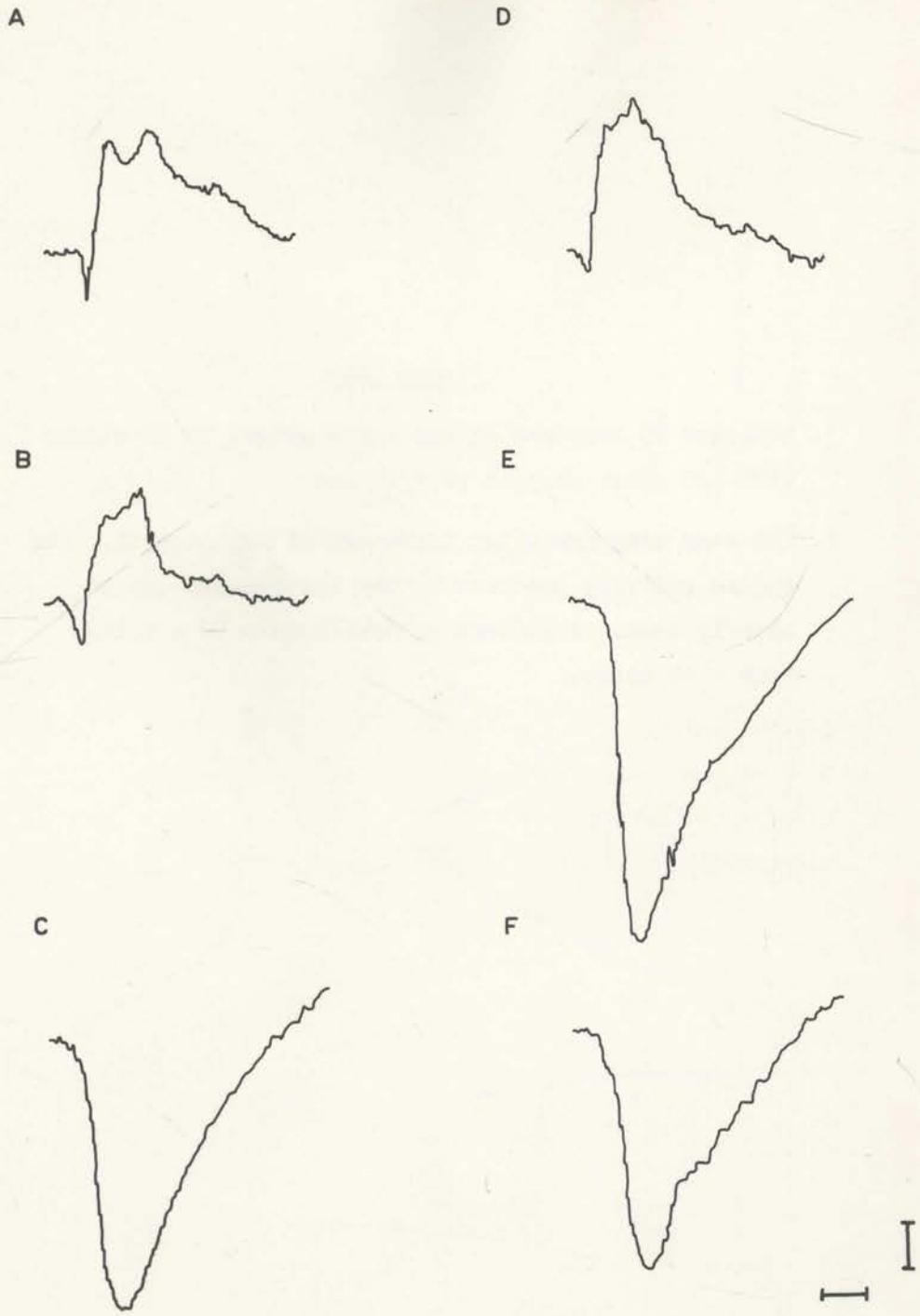

A
D
B
E
C
F

Figure 5-10

1. Averaged MG response of one awake animal to 50 clicks (105 db) given at rate of 1/10 sec.
2. The same response after injection of barbiturate. The cyclic activity produced by the barbiturate can be clearly seen. Amplitude calibrations = 50 μ volts, time = 50 msecs.

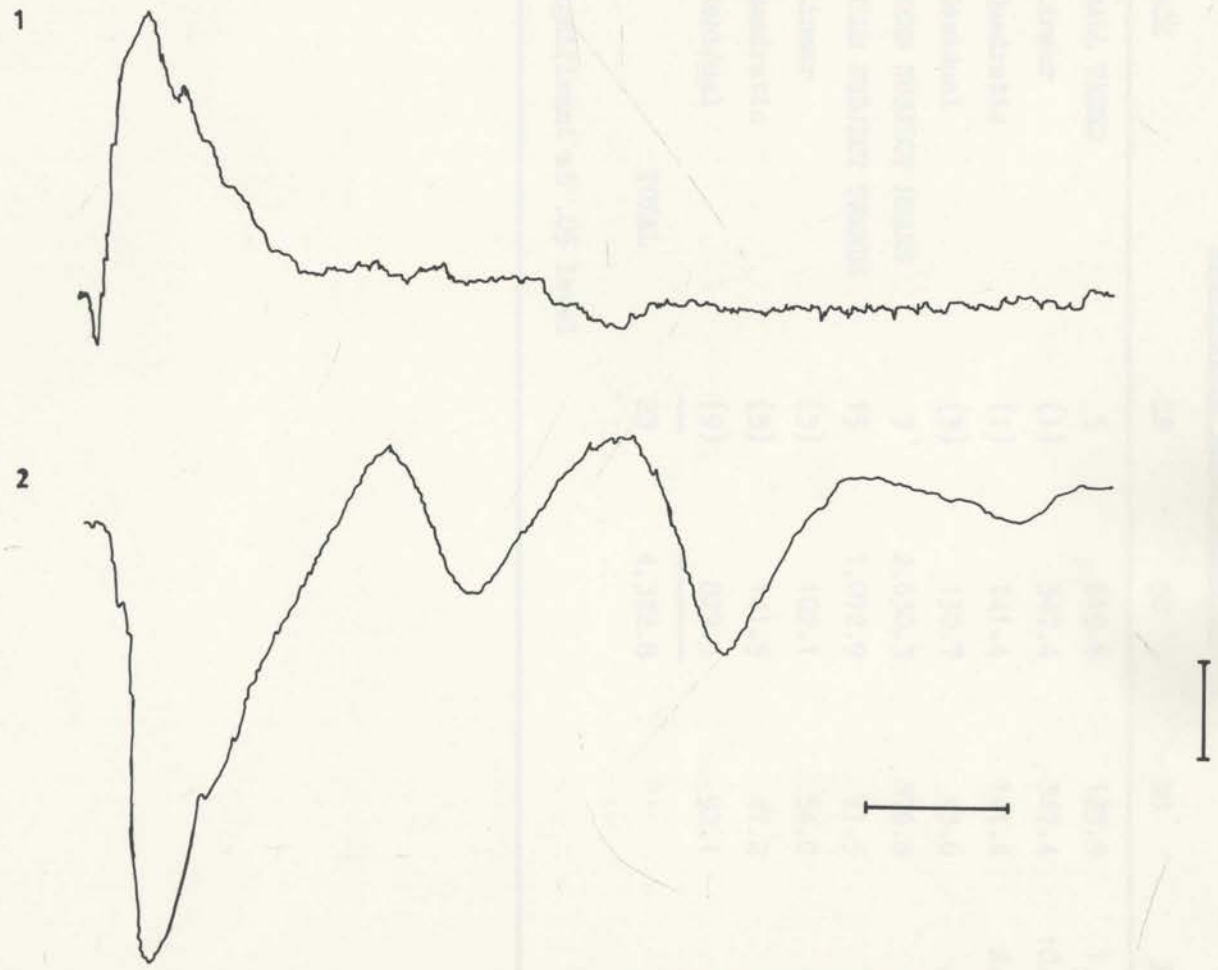

1
2

TABLE 27

SUMMARY OF ORTHOGONAL TREND ANALYSIS

Medial Geniculate - Experiment 9

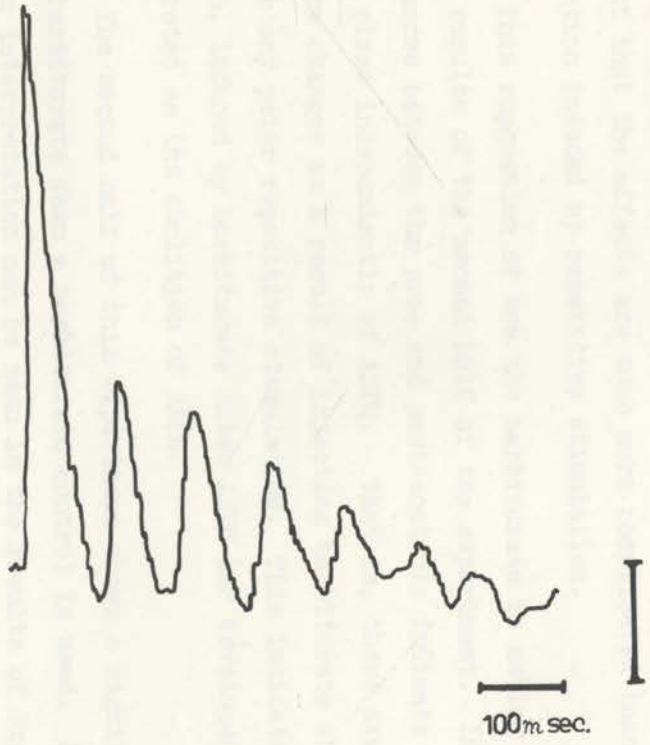
Habituation under Nembutal

SOURCE	DF	SS	MS	F
OVERALL TREND	5	619.6	123.9	1.73
Linear	(1)	347.4	347.4	10.20 *
Quadratic	(1)	141.4	141.4	2.99
Residual	(3)	130.7	43.6	.47
BETWEEN SUBJECT MEANS	3	2,630.3	876.8	
BETWEEN SUBJECT TRENDS	15	1,072.9	71.5	
Linear	(3)	102.1	34.0	
Quadratic	(3)	141.5	47.2	
Residual	(9)	829.3	92.1	
TOTAL	23	4,322.8		

* significant at .05 level

Figure 5-11

X-Y plot of averaged MG response to 50 105 db clicks given at a rate of 1/10 sec under barbiturate. The C.A.T. was set to average over 1,000 msecs. The marked cyclic activity occurring at intervals of approximately 100 msecs and gradually waning can be clearly seen. Amplitude calibration = 50 μ volts.


100m sec.

5.4.6 Discussion

The results of the first part of this experiment confirm the results of Experiments 7 and 8, in that barbiturates produce increases in the amplitude of MG evoked potentials after AEPH has developed. The averaged responses obtained from the C.A.T., however, show that there are clear-cut qualitative changes in some of the waveforms of the potentials. This confirms the hypothesis that barbiturate is doing more than producing a return to pre-habituation levels. It seems that barbiturate is acting directly on the nucleus. It is still possible that this change is mediated via the reticular formation, but even if this argument is accepted, it is apparent that the effects are much more complicated than the removal of habituation induced by repetitive stimulation.

This suggestion of how the barbiturate is acting is strongly supported by the results of the second half of the experiment. In particular, the differences between the pre- and post-controls indicate that changes are taking place independently of AEPH. That is, there are amplitude and waveform changes as a result of injecting barbiturate which are present without any prior repetitive stimulation. This indicates that similar changes, induced by barbiturate after AEPH has developed, cannot be interpreted as the abolition of AEPH.

The second half of this experiment shows a significant AEPH decrement under barbiturate when a barbiturate control is used. Further support for such an interpretation can be seen in the results of Experiment 8. Here it was observed that a rate effect was still present under barbiturate. This also suggests that despite the changes due to barbiturate per se, significant AEPH decrements are still present.

There are other features of the barbiturate changes which are important. Not only were amplitude changes observed, but in some placements there were drastic changes in waveform (Figures 5-10, 5-11). This consisted of the appearance of cyclic activity at intervals of approximately 100 msecs, which appear to be similar to the barbiturate spindles reported in the MG by Andersen et al(1967). These waveform differences do not appear to be correlated with placement, but this might be a function of the rather large size of the lesion made for histological identification. This problem will

be discussed in the context of later experiments.

It would seem that four main conclusions can be drawn from the results of Experiments 7, 8 and 9:

(1) The AEPH decrements at the CN and IC are not brought about by inhibition from the reticular formation, since the decrements are still present under barbiturate. The increased depression of response is similar to other observations concerning the action of barbiturates on sensory pathways (Chang, 1959).

(2) The AEPH decrements can occur independently of the state of arousal of the animal. This is indicated by the results obtained at the CN and IC. These barbiturate results suggest that the decrements are not solely a function of a particular arousal state.

(3) Changes occur at the MG which are a direct result of barbiturate and are independent of AEPH.

(4) The AEPH decrements can occur when the middle ear muscles are inactivated. It has been shown that surgical doses of barbiturate abolish the middle ear muscular reflex (Carmel and Starr, 1963), yet AEPH is still observed at the CN and the IC under barbiturate.

5.5 Experiment 10 - AEPH Under Flaxedil

5.5.1 Rationale and Aim

The aim of this experiment was to study the development of AEPH under a muscular paralysing drug, Flaxedil. Some of the possible actions of the middle ear muscles have been ruled out already by Experiments 6, 7, 8 and 9. Experiment 6 showed that the muscles were not acting through classical conditioning and the other experiments showed AEPH under a dose of barbiturate known to block the action of this reflex. However, to rule out the action of these muscles completely, it was decided to study the development of AEPH under Flaxedil.

5.5.2 Subjects, Stimulus Parameters and Procedures

Three placements already used in Experiment 3 were studied at the CN, IC and MG. The stimulus was an 85 db, 20 msec "click" presented through loudspeakers. A control record was first obtained with six stimuli presented at random. Then each animal was stimulated for 20 minutes with the clicks being presented at the rate of one per second. Each animal was tested without Flaxedil and then under Flaxedil.

To prepare for Flaxedil, they were anaesthetised with ether after an intraperitoneal injection of atropine (0.4 mg/Kg) to prevent excessive mucous secretion. The trachea and a radial vein were then cannulated. All wounds were infiltrated with Xylocaine (Astra) and sutured closed. The animals were placed in the test box and connected to a Palmer artificial respirator. An intravenous injection of Flaxedil (Gallamine Triethiodide, May & Baker, 40 mg/Kg) was given. Then the animal was continuously infused with Flaxedil (10 mg/Kg/hour) through a Palmer Slow Injection apparatus. Two hours were allowed for the ether to blow off before testing commenced. Further injections of Xylocaine were regularly given around the sutures to control possible pain.

At the end of each session, the rate of stimulation was changed to 1/10 sec, then to 10/1 sec and then back to 1/10 sec. This was done to see whether spontaneous recovery occurred when the rate of stimulation was reduced.

5.5.3 Data Reduction and Statistical Analysis

The data reduction was similar to that described in section 3.3.5. The results were analysed with the test of trend. Only the percentage data are reported. A type I and type II error rate of .05 was set.

5.5.4 Results

The measure was once again the peak-to-peak deflection occurring 250 msec after the stimulus. No attempt was made to analyse the components of the evoked potential. Figure 5-12 shows the percentage data plotted as a function of time. Figure 5-13 shows evoked potentials recorded with and without Flaxedil. It can be seen that AEPH has occurred when the animal is paralysed. The statistical results are as follows:

(a) Cochlear Nucleus

Table 28 summarises the results of the test of trend for the CN. There is a significant overall trend component ($p < .001$), but there is no difference between the two groups. This indicates that similar decrements occur both with and without Flaxedil (Figure 5-12).

(b) Inferior Colliculus

Table 29 summarises the results of the test of trend for the IC There is a significant overall trend component ($p < .001$), a significant overall linear component ($p < .01$) and a significant overall quadratic component ($p < .01$). There was no significant difference between the two groups. This indicates that the same degree of AEPH has developed with and without Flaxedil (Fig. 5-11).

(c) Medial Geniculate Body

Table 30 summarises the results of the test of trend for the MG. There is a significant overall trend component ($p < .001$) and a significant overall linear component ($p < .05$). There is no significant difference between the group trends but there is a significant difference between the group means ($p < .05$). Figure 5-12 shows that this is due to a greater decrement occurring under Flaxedil.

Figure 5-11 also shows that when the rate of stimulation is changed, then the amplitude of the evoked response at each nucleus is an inverse

TABLE 28

SUMMARY OF ORTHOGONAL TREND ANALYSIS

Cochlear Nucleus - Experiment 10

SOURCE	DF	SS	MS	F
OVERALL TREND	10	.0633	.0063	11.65 ***
Linear	(1)	.0030	.0030	1.16
Quadratic	(1)	.0091	.0091	17.44
Residual	(8)	.0513	.0064	21.33 ***
BETWEEN TREATMENT MEANS	1	.0066	.0066	.96
BETWEEN TREATMENT TRENDS	10	.0086	.0009	.59
Linear	(1)	.00004	.00004	.01
Quadratic	(1)	.0031	.0031	2.02
Residual	(8)	.0056	.0007	.77
BETWEEN SUBJECT MEANS	2	.0352	.0176	
SUBJECT BY TREATMENT MEANS	2	.0138	.0069	
BETWEEN SUBJECT TRENDS	20	.0109	.0005	
Linear	(2)	.0151	.0026	
Quadratic	(2)	.0010	.0005	
Residual	(16)	.0047	.0003	
SUBJECT BY TREATMENT TRENDS	20	.0291	.0015	
Linear	(2)	.0105	.0053	
Quadratic	(2)	.0030	.0015	
Residual	(16)	.0155	.0009	
TOTAL	65	.1675		

*** significant at .001 level

TABLE 29

SUMMARY OF ORTHOGONAL TREND ANALYSIS

Inferior Colliculus- Experiment 10

SOURCE	DF	SS	MS	F
OVERALL TREND	10	.3788	.0379	40.36 ***
Linear	(1)	.1767	.1767	126.22 **
Quadratic	(1)	.0535	.0535	108.88 **
Residual	(8)	.1487	.0186	20.66 **
BETWEEN TREATMENT MEANS	1	.0459	.0459	10.18
BETWEEN TREATMENT TRENDS	10	.0095	.0010	1.08
Linear	(1)	.0064	.0064	2.91
Quadratic	(1)	.0002	.0002	.46
Residual	(8)	.0030	.0004	.50
BETWEEN SUBJECT MEANS	2	.0301	.0150	
SUBJECT BY TREATMENT MEANS	2	.0090	.0045	
BETWEEN SUBJECT TRENDS	20	.0188	.0009	
Linear	(2)	.0028	.0014	
Quadratic	(2)	.0010	.0005	
Residual	(16)	.0151	.0009	
SUBJECT BY TREATMENT TRENDS	20	.0177	.0009	
Linear	(2)	.0044	.0022	
Quadratic	(2)	.0009	.0005	
Residual	(16)	.0124	.0008	
TOTAL	65	.5097		

*** significant at .001 level

** significant at .01 level

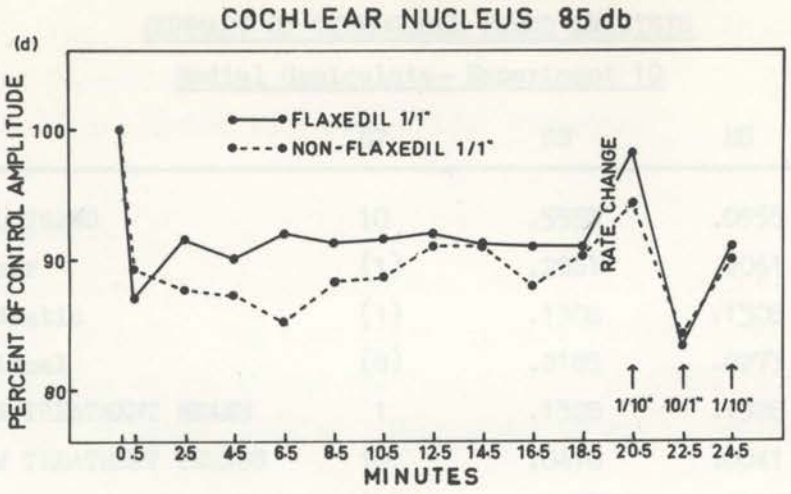
* significant at .05 level

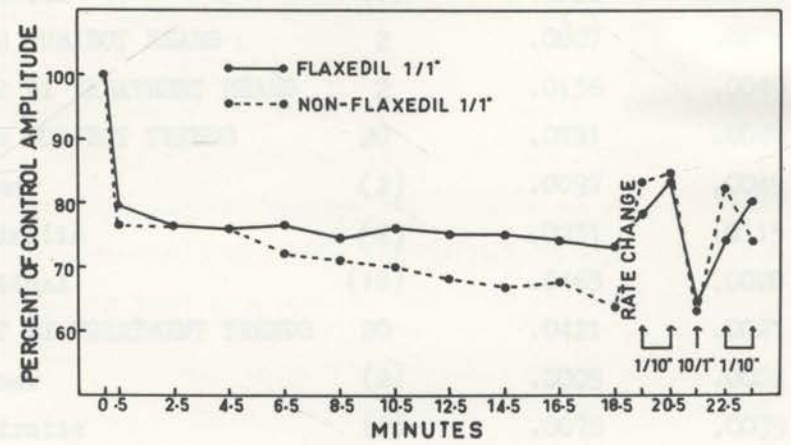
Figure 5-12

(a) The top graph shows percentage of control amplitude data plotted as a function of time for evoked potentials recorded at the CN. The stimulus was an 85 db click given at one per second. At the 20.5 minute mark, the rate was changed to one per ten seconds, then to ten per second and then back to one per ten seconds. The parameters are Flaxedil and non-Flaxedil.

(b) The middle graph shows similar data for the IC.

(c) The bottom graph shows similar data for the MG.


COCHLEAR NUCLEUS 85 db
(d)
PERCENT OF CONTROL AMPLITUDE
100
90
80
FLAXEDIL 1/1"
NON-FLAXEDIL 1/1"
RATE CHANGE
1/10"
10/1"
1/10"
0·5
2·5
4·5
6·5
8·5
10·5
12·5
14·5
16·5
18·5
20·5
22·5
24·5
MINUTES


INFERIOR COLLICULUS 85db
PERCENT OF CONTROL AMPLITUDE
100
90
80
70
60
FLAXEDIL 1/1"
NON-FLAXEDIL 1/1"
RATE CHANGE
1/10"
10/1"
1/10"
0·5
2·5
4·5
6·5
8·5
10·5
12·5
14·5
16·5
18·5
20·5
22·5
MINUTES

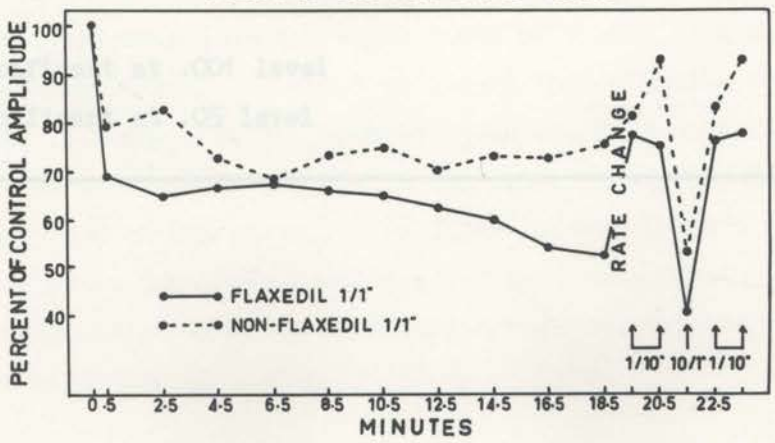

MEDIAL GENICULATE 85db
PERCENT OF CONTROL AMPLITUDE
100
90
80
70
60
50
40
FLAXEDIL 1/1"
NON-FLAXEDIL 1/1"
RATE CHANGE
1/10"
10/1"
1/10"
0·5
2·5
4·5
6·5
8·5
10·5
12·5
14·5
16·5
18·5
20·5
22·5
MINUTES

TABLE 30

SUMMARY OF OTHROGONAL TREND ANALYSIS

Medial Geniculate - Experiment 10

SOURCE	DF	SS	MS	F	
OVERALL TREND	10	.5555	.0555	14.05	***
Linear	(1)	.2061	.2061	42.46	*
Quadratic	(1)	.1308	.1308	11.33	
Residual	(8)	.2188	.0273	9.71	***
BETWEEN TREATMENT MEANS	1	.1328	.1328	19.57	*
BETWEEN TREATMENT TRENDS	10	.0410	.0041	1.95	
Linear	(1)	.0046	.0046	17.89	
Quadratic	(1)	.0011	.0011	.27	
Residual	(8)	.0355	.0044	2.09	
BETWEEN SUBJECT MEANS	2	.0807	.0403		
SUBJECT BY TREATMENT MEANS	2	.0136	.0049		
BETWEEN SUBJECT TRENDS	20	.0791	.0040		
Linear	(2)	.0097	.0049		
Quadratic	(2)	.0231	.0115		
Residual	(16)	.0463	.0028		
SUBJECT BY TREATMENT TRENDS	20	.0421	.0021		
Linear	(2)	.0005	.0002		
Quadratic	(2)	.0078	.0039		
Residual	(16)	.0338	.0021		
TOTAL	65	.9447			

*** significant at .001 level

* significant at .05 level

function of the rate of stimulation. When the rate of stimulation is increased, then the amplitude decreases. The same effects are obtained with and without Flaxedil. Figure 5-13 shows evoked potentials recorded from each of the three nuclei with and without Flaxedil.

5.5.5 Discussion

The results of this experiment show that middle ear muscles are not the determinants of AEPH. The same degree of AEPH occurs under Flaxedil as without the drug at the CN and the IC. At the MG, there is significantly more habituation under Flaxedil. This might be due to central effects of Flaxedil (Halpern and Black, 1967), but there is no available evidence for a selective action on the thalamus. While this result is interesting in itself, it still leaves unchallenged the main result of this experiment. This is that a significant decrement occurs under Flaxedil at all three nuclei.

The observation that the amplitude of the evoked potential is inversely related to rate of stimulation, both with and without Flaxedil, has important implications. It indicates that the effects of stimulating at ten per one second in Experiment 3 were not determined by middle ear muscular activity. It also suggests that spontaneous recovery of AEPH would occur if stimulation were stopped.

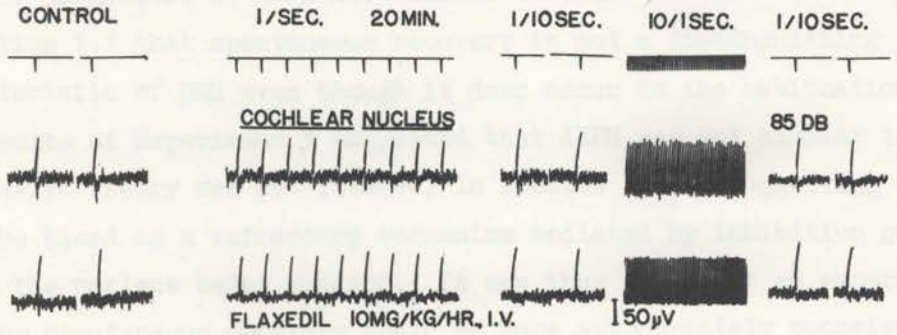
Both of these results are compatible with the theory of intrinsic inhibition which, in effect, says that AEPH is due to a refractory mechanism. However, both of these observations are difficult for any theory that AEPH is due to learning. It would be difficult for a learning explanation to account for this relationship, which seems more akin to a dissipation effect than a learning effect. It could be argued that stimulus change per se has reduced the learning effect. However, this would be equivalent to arguing that the interval between stimuli is the conditioned stimulus for not responding. But it has already been shown in Experiment 6 that a regular interval is not a necessary condition for the development of AEPH. When taken in conjunction with the barbiturate results, this experiment makes a learning explanation untenable.

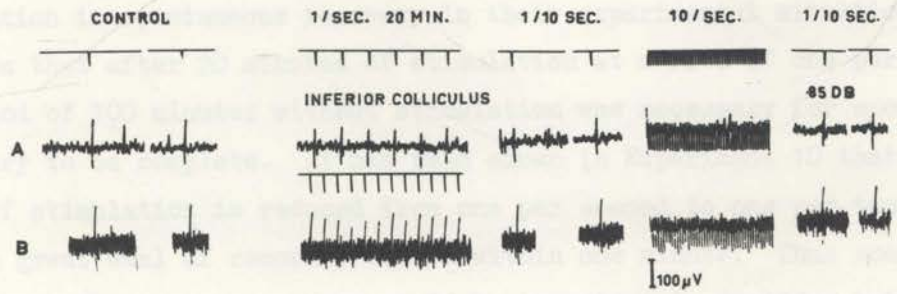
Figure 5-13

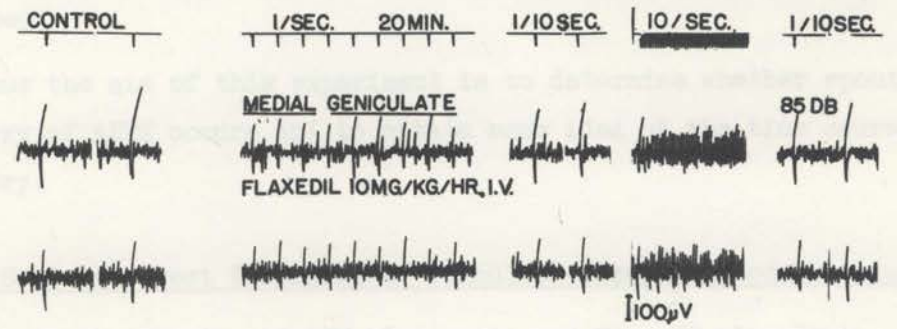
(a) The top two records are evoked potentials recorded from the CN. The first row is recorded from an awake, unrestrained cat. The second row is recorded from the same animal paralysed with Flaxedil. The first two evoked potentials are control levels, followed by a sample after 20 minutes stimulation at one per second. A number of rate changes are then shown.

(b) The middle two traces are from the IC. The first is without Flaxedil and the second is recorded under Flaxedil.

(c) Similar evoked potential records for the MG.


CONTROL
1/SEC.
20MIN.
1/10SEC.
10/1SEC.
1/10SEC.
COCHLEAR NUCLEUS
85 DB
FLAXEDIL 10MG/KG/HR, I.V.
50μV


CONTROL
1/SEC. 20 MIN.
1/10 SEC.
10/SEC.
1/10 SEC.
INFERIOR COLLICULUS
85 DB
A
B
100μV


CONTROL
1/SEC.
20MIN.
1/10SEC.
10/SEC.
1/10SEC.
MEDIAL GENICULATE
85 DB
FLAXEDIL 10MG/KG/HR, I.V.
100μV

5.6 Experiment 11 - Spontaneous Recovery of AEPH

5.6.1 Rationale and Aim

This experiment was carried out after Experiment 4. It was originally conceived as one of a series of experiments investigating the proposition that the parameters of AEPH are similar to those of BRH. It was pointed out in section 1.1 that spontaneous recovery is not a distinguishing characteristic of BRH even though it does occur in the habituation situation. The results of Experiment 3 suggested that AEPH was not similar to BRH and a tentative theory was put forward, in section 3.3.8, suggesting that AEPH might be based on a refractory mechanism mediated by inhibition generated within the nucleus being studied. It was thus felt that an experiment studying spontaneous recovery would be more appropriately conceived of as a possible test of the various theories of AEPH.

Thompson and Spencer (1966) have argued against the role of synaptic inhibition in spontaneous recovery in their experimental situation, on the grounds that after 20 minutes of stimulation at a rate of one per second, a period of 100 minutes without stimulation was necessary for spontaneous recovery to be complete. It has been shown in Experiment 10 that when the rate of stimulation is reduced from one per second to one per ten seconds that a great deal of recovery occurs within one minute. Thus some idea of how long AEPH takes to recover would be important in deciding between the various theories. It would be expected on the basis of Experiment 3 that recovery would be rapid since no decrements occurred to stimulation at 1/20 sec.

Thus the aim of this experiment is to determine whether spontaneous recovery of AEPH occurs and to obtain some idea of the time course of recovery.

5.6.2 Subjects, Test Environment, Stimulus Parameters and Procedure

Six CN placements, six MG placements and four IC placements were used from the animals implanted for Experiment 3 (section 3.3.2). The test environment was the same as employed in that experiment (section 3.3.4). The stimulus was a 20 msec "click" given at 85 db re .0002 microbar. Three

rates of stimulation were employed: 1/10 sec, 1/1 sec, and 10/1 sec. The stimuli were delivered through loudspeakers and generated and measured as described in section 3.2.3.

The procedure was as follows: each animal was tested once under each of the three stimulus conditions. A control level was obtained from six stimuli given at random intervals with no one interval being less than 30 seconds. This was followed by 20 minutes stimulation under one of the three conditions. At the end of 20 minutes, stimulation ceased and four random test stimuli were given every five minutes for a further 30 minutes to obtain a recovery function. The interval between each test stimulus was never less than 20 seconds.

5.6.3 Data Reduction and Statistical Analysis

The data were measured in the way set out in section 3.3.5. There were three stages in the statistical analysis:

(1) A test of trend was carried out on the pre-recovery data to determine whether a decrement had occurred.

(2) A test of trend was carried out on the recovery data to determine differences between recovery under the different stimulus conditions.

(3) A t test was carried out on the first recovery point to see whether complete recovery had occurred in the first five minutes.

A type I and type II error rate of .05 were set. It was predicted that there would be a rate effect in the pre-recovery data and no rate effect in the recovery data. On the basis of Experiment 5, it was expected that recovery would be virtually complete in the first five minutes.

5.6.4 Results

(a) Cochlear Nucleus

The test of trend for the recovery data is summarised in Table 31. The percentage data plotted as a function of time are seen in Figure 5-14. The analysis shows that significant habituation has occurred over the three conditions (overall trend, $p < .01$; linear component, $p < .05$). There is a significant difference between group means ($p < .01$) and between group trends ($p < .001$). None of the components of between group trends reach significance.

TABLE 31

(1) SUMMARY OF ORTHOGONAL TREND ANALYSIS OF PRE-RECOVERY DATA
Cochlear Nucleus - Experiment 11

SOURCE	DF	SS	MS	F	
OVERALL TREND	10	.3464	.0346	5.94	**
Linear	(1)	.0841	.0841	12.15	*
Quadratic	(1)	.0293	.0293	1.71	
Residual	(8)	.2332	.0291	6.93	***
BETWEEN TREATMENT MEANS	2	1.4872	.7436	13.85	**
BETWEEN TREATMENT TRENDS	20	.2128	.0106	4.09	***
Linear	(2)	.0402	.0201	3.69	
Quadratic	(2)	.0307	.0154	2.42	
Residual	(16)	.1420	.0088	5.18	***
BETWEEN SUBJECT MEANS	5	2.0853	.4171		
SUBJECT BY TREATMENT MEANS	10	.5371	.0537		
BETWEEN SUBJECT TRENDS	50	.2916	.0058		
Linear	(5)	.0345	.0069		
Quadratic	(5)	.0855	.0171		
Residual	(40)	.1715	.0042		
SUBJECT BY TREATMENT TRENDS	100	.2600	.0026		
Linear	(10)	.0544	.0054		
Quadratic	(10)	.0636	.0064		
Residual	(80)	.1420	.0017		
TOTAL	197	5.2204			

*** significant at .001 level
** significant at .01 level
* significant at .05 level

(2) SUMMARY OF ORTHOGONAL TREND ANALYSIS OF RECOVERY DATA

OVERALL TREND	5	.0222	.0044	.82
Linear	(1)	.0156	.0156	2.36
Quadratic	(1)	.00004	.00004	.01
Residual	(3)	.0066	.0022	.43
BETWEEN TREATMENT MEANS	2	.0499	.0250	.80
BETWEEN TREATMENT TRENDS	10	.0420	.0042	.73
Linear	(2)	.0152	.0076	.73
Quadratic	(2)	.0021	.0011	.34
Residual	(6)	.0247	.0016	.08
BETWEEN SUBJECT MEANS	5	.5119	.1024	
SUBJECT BY TREATMENT MEANS	10	.3118	.0312	
BETWEEN SUBJECT TREND	25	.1345	.0054	
Linear	(5)	.0331	.0066	
Quadratic	(5)	.0240	.0048	
Residual	(15)	.0774	.0051	
SUBJECT BY TREATMENT TRENDS	50	.2868	.0057	
Linear	(10)	.1031	.0103	
Quadratic	(10)	.0317	.0032	
Residual	(30)	.1520	.0202	
TOTAL	107	1.3590		

(3) t-tests
(1) 85/10/1 VS 100 percent: $t = .61$, df 5, $p > .05$
(2) 85/1/1 VS 100 percent: $t = 2.82$, df 5, $p > .05$
(3) 85/1/10 VS 100 percent: $t = .79$, df 5, $p > .05$

These results show that there is a difference between the groups due to rate of stimulation.

The test of trend on the recovery data is summarised in the bottom half of Table 31, and the percentage recovery data plotted as a function of time are seen in the second half of Figure 5-14. The analysis shows that the curves do not differ from one another and do not differ from zero slope, since none of the trend or mean components are significant. The t-tests show no significant difference between the means of the first five minutes and 100 percent. That is, recovery appears to have been achieved in five minutes.

(b) Inferior Colliculus

The test of trend for the pre-recovery data is summarised in the top half of Table 32 and the data are plotted as a function of time in Figure 5-14. The analysis shows that overall habituation has occurred (overall trend, $p < .001$; linear component, $p < .05$). There is a significant difference between the group trends ($p < .05$). These results show that there is a difference between the groups due to rate of stimulation. The test of trend for the recovery data is summarised in the bottom half of Table 32 and in Figure 5-14. This shows that the recovery curves do not differ from one another and do not differ from zero slope. The t-tests show no significant difference between the means of the first five minutes and 100 percent. That is, recovery appears to have been achieved in five minutes.

(c) Medial Geniculate Body

The results of the pre-recovery test of trend are summarised in the top half of Table 33, and the data are plotted as a function of time in Figure 5-14. The analysis shows that significant overall habituation has occurred. (overall trend, $p < .001$; linear component, $p < .01$; quadratic, $p < .05$). There is a significant difference between group means ($p < .001$), between group trends ($p < .001$) and between groups linear trend ($p < .01$). This indicates that there is a significant effect due to rate of stimulation.

The recovery test of trend is summarised in the bottom half of Table 33, and it shows that there is no significant difference between conditions and no difference from zero slope. The t-tests show no difference between the

TABLE 32

(1) SUMMARY OF ORTHOGONAL TREND ANALYSIS FOR PRE-RECOVERY DATA
Inferior Colliculus - Experiment 11

SOURCE	DF	SS	MS	F
OVERALL TREND	10	.5228	.0523	9.09 ***
Linear	(1)	.2060	.2060	20.11 *
Quadratic	(1)	.0962	.0962	8.16
Residual	(8)	.2207	.0276	6.27 **
BETWEEN TREATMENT MEANS	3	.9787	.3262	2.83
BETWEEN TREATMENT TRENDS	20	.1774	.0089	2.04 *
Linear	(2)	.0362	.0181	2.49
Quadratic	(2)	.0509	.0254	2.54
Residual	(16)	.0827	.0051	1.59
BETWEEN SUBJECT MEANS	3	.9787	.3262	
SUBJECT BY TREATMENT MEANS	6	1.3078	.2180	
BETWEEN SUBJECT TRENDS	30	.1726	.0058	
Linear	(3)	.0307	.0102	
Quadratic	(3)	.0354	.0118	
Residual	(24)	.1065	.0044	
SUBJECT BY TREATMENT TRENDS	60	.2608	.0043	
Linear	(6)	.0436	.0073	
Quadratic	(6)	.0599	.0099	
Residual	(48)	.1573	.0032	
TOTAL	131	4.6555		

(2) SUMMARY OF ORTHOGONAL TREND ANALYSIS FOR RECOVERY DATA

OVERALL TREND	5	.0206	.0041	1.20
Linear	(1)	.0122	.0122	5.11
Quadratic	(1)	.0008	.0008	.09
Residual	(3)	.0078	.0026	1.26
BETWEEN TREATMENT MEANS	2	.1005	.0503	2.40
BETWEEN TREATMENT TRENDS	10	.0720	.0072	.61
Linear	(2)	.0231	.0115	.40
Quadratic	(2)	.0259	.0130	.95
Residual	(6)	.0231	.0038	.70
BETWEEN SUBJECT MEANS	3	.0530	.0180	
SUBJECT BY TREATMENT MEANS	6	.1256	.0209	
BETWEEN SUBJECT TRENDS	15	.0514	.0034	
Linear	(3)	.0071	.0024	
Quadratic	(3)	.0268	.0089	
Residual	(9)	.0176	.0019	
SUBJECT BY TREATMENT TRENDS	30	.3540	.0118	
Linear	(6)	.1742	.0290	
Quadratic	(6)	.0816	.0136	
Residual	(18)	.0984	.0054	
TOTAL	71	.7772		

(3) t-tests

(1) 85/10/1 VS 100 percent: $t = 3.1$, $df = 3$, $p > .05$
(2) 85/1/1 VS 100 percent: $t = 1.6$, $df = 3$, $p > .05$
(3) 85/1/10 VS 100 percent: $t = .80$, $df = 3$, $p > .05$

TABLE 33

(1) SUMMARY OF ORTHOGONAL TREND ANALYSIS
Medial Geniculate - Experiment 11

SOURCE	DF	SS	MS	F	
OVERALL TREND	10	.9260	.0926	16.40	***
Linear	(1)	.3496	.3496	30.47	**
Quadratic	(1)	.1950	.1950	18.11	*
Residual	(8)	.3814	.0476	11.33	***
BETWEEN TREATMENT MEANS	2	3.7261	1.8630	20.03	***
BETWEEN TREATMENT TRENDS	20	.4501	.0225	4.44	***
Linear	(2)	.0654	.0327	9.45	**
Quadratic	(2)	.0761	.0381	3.36	
Residual	(16)	.3086	.0192	3.91	***
BETWEEN SUBJECT MEANS	4	.5510	.1377		
SUBJECT BY TREATMENT MEANS	8	.7441	.0930		
BETWEEN SUBJECT TRENDS	40	.2259	.0056		
Linear	(4)	.0459	.0115		
Quadratic	(4)	.0431	.0108		
Residual	(32)	.1370	.0042		
SUBJECT BY TREATMENT TRENDS	80	.4055	.0051		
Linear	(8)	.0277	.0035		
Quadratic	(8)	.0905	.0113		
Residual	(64)	.2874	.0049		
	164	7.0286			

(2) SUMMARY OF ORTHOGONAL TREND ANALYSIS FOR RECOVERY DATA

OVERALL TREND	5	.0204	.0041	.39
Linear	(1)	.0026	.0026	.19
Quadratic	(1)	.0008	.0008	.07
Residual	(3)	.0170	.0056	.62
BETWEEN TREATMENT MEANS	2	.1041	.0520	.63
BETWEEN TREATMENT TRENDS	10	.1028	.0103	.75
Linear	(2)	.0504	.0252	1.80
Quadratic	(2)	.0271	.0135	4.27
Residual	(6)	.0254	.0042	.33
BETWEEN SUBJECT MEANS	4	.2802	.0700	
SUBJECT BY TREATMENT MEANS	8	.6620	.0827	
BETWEEN SUBJECT TRENDS	20	.2083	.0104	
Linear	(4)	.0558	.0140	
Quadratic	(4)	.0439	.011	
Residual	(12)	.1085	.0090	
SUBJECT BY TREATMENT TRENDS	40	.5445	.0136	
Linear	(8)	.2014	.0252	
Quadratic	(8)	.0330	.0041	
Residual	(24)	.3102	.0129	
TOTAL	89	1.9223		

(3) t-tests

(1) 85/10/1 VS 100 percent, $t = 2.35$, df 4, $p > .05$
(2) 85/1/1 VS 100 percent, $t = .06$, df 4, $p > .05$
(3) 85/1/10 VS 100 percent, $t = 1.9$, df 4, $p > .05$

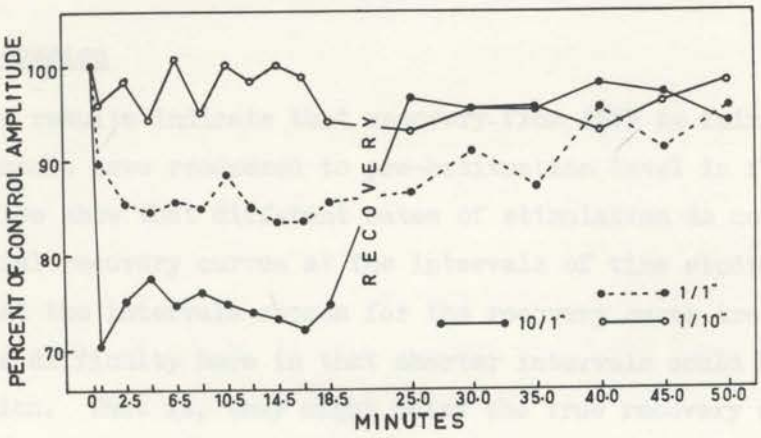
Figure 5-14

(a) The top graph shows percentage of control data plotted as a function of time for evoked potentials recorded at the CN. This graph shows the effects of repetitive stimulation with clicks of 85 db for 20 minutes. Then a spontaneous recovery curve is plotted for 30 minutes. The parameters are rates of ten per second, one per second, one per ten second.

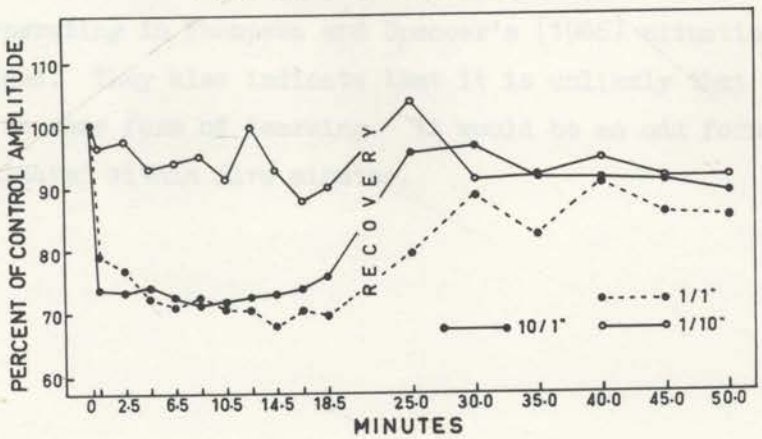
(b) The middle graph shows similar curves for the IC.

(c) The bottom graph shows similar curves for the MG.

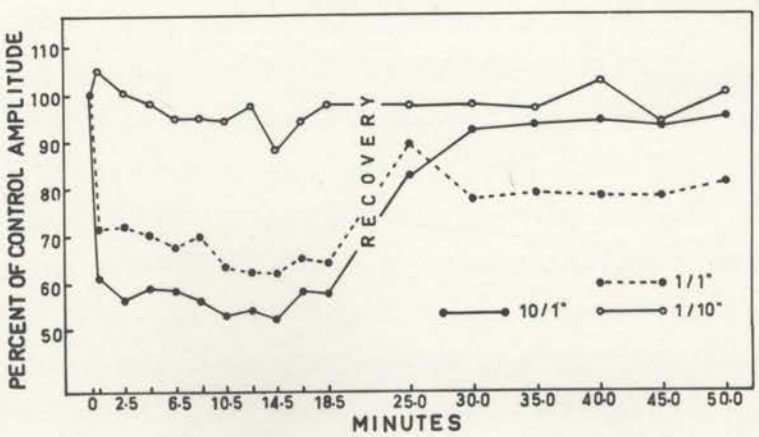
COCHLEAR NUCLEUS 85 db



INFERIOR COLLICULUS 85 db



MEDIAL GENICULATE 85 db



means of the first five minutes and 100 percent. This indicates that recovery appears to have been achieved in five minutes.

5.6.5 Discussion

These results indicate that recovery from AEPH is fairly rapid in that the placements have recovered to pre-habituation level in five minutes. The results also show that different rates of stimulation do not produce differential recovery curves at the intervals of time studied. It is most likely that the intervals chosen for the recovery curve are too large, but there is a difficulty here in that shorter intervals could have still produced a depression. That is, they might delay the true recovery curve.

These results are in keeping with the theory that AEPH is due to synaptic inhibition and they suggest that the processes operating here are different to those operating in Thompson and Spencer's (1966) situation where recovery was prolonged. They also indicate that it is unlikely that the decrements are based on some form of learning. It would be an odd form of learning which dissipated within five minutes.

5.7 General Discussion

The aim of this series of experiments has been to test the various theories of AEPH outlined in Chapter 4.0. In this discussion, the evidence related to each theory will be drawn together and evaluated.

(a) AEPH as a function of conditioning the middle ear muscles:

This theory has been ruled out on the basis of four pieces of evidence. Firstly, AEPH occurs when stimuli are presented with random intervals between them. The theory argues that the response of the muscles becomes conditioned to the regular stimulus interval. There is no way that the muscles could become so conditioned when the inter-stimulus interval varies randomly. Secondly, the results of Experiment 8 show that when a random set of intervals is used, the amplitude of the potential evoked by a stimulus is directly related to the time since the last stimulus. This observation is impossible to explain in terms of classical conditioning. Thirdly, the fact that AEPH occurs under a dose of barbiturate that is known to block the action of the middle ear muscles also rules out their participation. Fourthly, AEPH occurs under Flaxedil which paralyses these muscles. All of these results refute the theory of conditioning of the middle ear muscles. The last two observations also exclude the possibility that the middle ear muscles have a tonic action which produces AEPH.

(b) Afferent neuronal inhibition

This theory has been ruled out on the basis of the experiments using barbiturates. At the CN and the IC, it is apparent that barbiturates do not abolish AEPH. The afferent neuronal inhibition theory predicts that surgical doses of barbiturates would abolish AEPH. At the MG, the results are more complex. There is an apparent tendency for the evoked potential amplitude to return to pre-AEPH levels. However, two pieces of evidence suggest that these results are not compatible with afferent neuronal inhibition. Firstly, after injection there is often a qualitative change in the waveform recorded at the MG. This suggests that the change is more than a mere return to pre-AEPH levels and hence an abolition of AEPH. Secondly, in Experiment 9 it was observed that there was an increase in peak-to-peak amplitude when a pre-anaesthetic control was compared with a post-anaesthetic control. Since

this increase occurred in the absence of AEPH, it is difficult to argue that any increase which occurs as a result of an injection after AEPH has developed, is in fact an abolition of the inhibition producing AEPH.

In addition, the evidence from Experiment 9 indicating that AEPH develops after injection of barbiturates is very difficult for the theory of afferent neuronal inhibition. This evidence indicates that despite the changes produced by barbiturates, there is a decrement to repetitive stimulation. Whilst all this evidence concerning the MG suggests that AEPH is not due to inhibition from the reticular formation, it does not rule out the possibility that the reticular formation influences the area. For example, the qualitative waveform changes could be due to the removal of reticular formation influence. These experiments do not bear on this issue, but one interesting point is that the qualitative changes of waveform are very similar to rhythmic spindling reported by Andersen et al (1967) in the MG, who have produced evidence which suggests that this spindling is intrinsic to the thalamus.

(c) Cortical inhibition

This theory has not been tested directly but it has been weakened considerably by two pieces of evidence. Firstly, the evidence that barbiturates do not abolish AEPH is a difficult observation for this theory to explain. Desmedt (1960) has postulated that the temporo-insular cortex is the site of the inhibition which produces AEPH. Thompson (1967) has shown that barbiturates have a marked effect on the activity of such association areas of the cortex. Thus it is surprising, even if it is not crucial, that decrements at the CN and IC persist under barbiturate. It is difficult to imagine how this theory could explain this effect. Secondly, the changes produced by altering the rate of stimulation in Experiment 10 are difficult for this theory to explain. If one regards the change of rate as being a novel stimulus, then this theory would predict that a change in rate would lead to dishabituation. The fact that the decrement is a direct function of rate of stimulation poses a problem for this theory in its present form.

(d) AEPH as a form of learning

There is no existing theory involving an explanation of AEPH in terms of

learning. There is a theory that BRH is based on learning, and, by analogy, AEPH could be considered in this way. The evidence that AEPH is a function of rate of stimulation does not fit into a learning model. Also the evidence that the degree of AEPH can be decreased or increased by a change in rate of stimulation is hardly in keeping with any definition of learning. This type of theory will not be considered further in this dissertation.

(e) Intrinsic inhibition theory

This theory has been put forward in this thesis to explain the phenomenon of AEPH. It argues that AEPH is based on a refractory mechanism in the form of an inhibitory process in the particular nucleus of the auditory pathway under investigation. This theoretical position was suggested by parametric studies in this thesis and by the observations that there are relatively long-lasting inhibitory processes in the central nervous system (Bishop, 1964; Burke and Sefton, 1966). Thompson and Spencer (1966) rejected any explanation of this type as accounting for habituation in their acute spinal preparation, on the basis of three pieces of evidence. Firstly, they pointed out that the known types of pre- and post-synaptic inhibition in the central nervous system had time courses of less than 500 msecs (they did not take into account the work of Bishop, Burke and colleagues mentioned above), and argued that this time course is too short even to explain habituation to stimuli given at the rate of one per second. Secondly, not only is the time course too short, but it would need to summate to produce the effect. In addition, the summation would need to be of a long-lasting depressive character. For example, in their experiments they find that stimulating at a rate of one per second for 20 minutes, that spontaneous recovery takes about 100 minutes, which is a very long-lasting depression of responding. The third piece of evidence is that pharmacological studies with their preparation do not support the notion of BRH being based on synaptic inhibition.

The third point is valid for their preparation, but it might not be relevant to the auditory pathway. This is a weak rejoinder to their criticism, but the other two points seem more important in the present context. The observations of Bishop, Burke and colleagues that inhibition in the lateral geniculate can last at least five seconds, and in some cases up to 10 - 12

seconds (personal communication from W.R. Burke), suggests that intrinsic inhibition is a plausible mechanism. Secondly, spontaneous recovery with AEPH is very much faster than the recovery reported by Thompson and Spencer (1966). The experiment on spontaneous recovery is not sensitive enough to show whether the recovery curve is similar to those of Sefton and Burke (1965), but it does indicate that recovery is complete in about five minutes (this point will be further investigated in later experiments). Thus it seems plausible that AEPH could be based on synaptic inhibition.

The results of experiments with barbiturates are generally in line with expectations from this theory, in particular the fact that barbiturate enhances decrements at the CN and IC. The MG evidence is difficult to interpret in relation to this position and will need further testing. Also, the effects of random stimuli both with and without barbiturate are also in keeping with intrinsic inhibition. Moreover, the observation that degree of AEPH is directly related to the direction of change of rate of stimulation is in accord with this theory. When the rate is increased, the degree of response decrement is increased and vice versa.

All of these observations give indirect support for the theory, and in a sense give further weight to it because they are extremely difficult for other theories to interpret. There is one consequence of the intrinsic inhibition theory that must be mentioned at this stage. If this theory of AEPH is true, then is AEPH, as defined by experiments reported here, really an habituation phenomenon? It has already been shown in the parametric studies that AEPH does not follow the same parameters as BRH. If it turns out that AEPH is based on a type of refractory process, then does it make any sense to call it habituation? It may be a refractory process which has been confused with an habituation process. As mentioned in section 1.1.4, it is possible for a link in an afferent-efferent chain to contribute to habituation without showing all the parameters of BRH. Thus the auditory pathway could contribute toward the rate effect observed by Hunter and Prosser (1936) without contributing toward any other feature of habituation.

It may be that Thompson and Spencer (1966) are correct in their conclusion that sensory habituation has not been demonstrated. But the reason could be that what was thought to be habituation, at least in the auditory

pathway, has been confused with another process, a process which might or might not contribute to habituation. Thus the conclusion of this chapter is that AEPH could be based on synaptic inhibition intrinsic to a particular nucleus; and furthermore, if this is true, that AEPH should not properly be called an habituation process. The experiments described in the next chapter will further endeavour to determine whether the observed evoked potential depressions may be attributed to a type of refractory process based on synaptic inhibition.

CHAPTER 6

6.0 Experiments on Intrinsic Inhibition

It seems fairly clear that only the theory of intrinsic inhibition remains unchallenged by the experiments testing the various theories. However, in none of the experiments has this theory been directly tested. It is extremely difficult to test directly a theory about synaptic inhibition using the evoked potential technique and physiological stimuli. Yet it would seem that further indirect tests might give some support to the plausibility of this theory.

One implication of the theory is that the time since the last stimulus should largely determine the size of the evoked potential to the next stimulus. Not one of the other theories can make this prediction as they stand at present. It would appear then that an analysis of the recovery function of the CN, IC and MG would be one way to test this theory.

6.1 Experiment 13 - Amplitude Recovery - I

We have seen that there is an increasing decrement in evoked potential amplitude to repetitive stimulation as the interstimulus interval is reduced to 100 msecs. The decrement is not less under Flaxedil, and this observation suggests that the middle ear muscles are not a significant factor. However, it is possible that the muscles might be involved if intervals shorter than 100 msec were employed. It would seem that some control over their action would be required before a test of synaptic effects could be attempted.

Furthermore, the theory of synaptic inhibition would predict that there would be slower recovery under barbiturate compared with the awake animal. Thus the aim of this experiment was to study the recovery of evoked potential responsiveness at the CN, IC and MG under two conditions:

(1) under muscular paralysis induced by Flaxedil

(2) after injection of barbiturate and also under muscular paralysis induced by Flaxedil.

6.1.2 Subjects and Electrode Implantation

Six adult cats were anaesthetised with ether after an intraperitoneal injection of atropine (0.4 mg/Kg) to prevent excessive mucous secretion. The trachea and a radial vein were cannulated. The animals were then placed in a Trent Wells stereotaxic machine and the cortex exposed so that electrodes could be lowered stereotaxically into the CN, IC and MG. All wounds were then infiltrated with Xylocaine (Astra). The stereotaxic instrument and the animal were then placed in a shielded test box and connected to a Palmer artificial respirator. An intravenous injection of Flaxedil (Gallamine triethiodide, May & Baker, 40 mg/Kg) was given. The animal was then continuously infused by Flaxedil (10 mg/Kg/hour) through a Palmer Slow Injection Apparatus. Two hours were allowed for the ether to blow off before testing commenced. Regular injections of Xylocaine were given to control pain around the sutures. Earphones (AKG-50) were attached to hollow ear bars. Bipolar electrodes were lowered into position in each nucleus while monitoring auditory stimuli were given.

6.1.3 Stimulus Parameters and Procedures

The stimulus was an 85 db re .0002 microbar, 20 msec click. The procedure was as follows:

(a) A control condition was obtained by giving clicks at the rate of one per five second.

(b) A sample of evoked potentials was obtained with the following intervals given in a random order: 1000, 500, 250, 100, 80, 50, 40, 32 msecs. At least five minutes were allowed to elapse after each brief presentation of the sequence of about 30 stimuli.

(c) The same procedure was followed after the animals were given an intravenous injection of barbiturate (30 mg/Kg Nembutal, Abbott).

6.1.4 Recording System and Data Reduction

The evoked responses were photographed by a Grass Kymograph camera on an RM 561 Tektronix Oscilloscope. For the control condition and the 1000 msec interval, individual frames were photographed. For all other intervals,

the oscilloscope time base was stopped and the film moved in the camera.

The resulting film was projected through an enlarger onto graph paper and the peak-to-peak amplitude measured. With intervals of 500 msec and shorter, the first evoked potential was ignored as it obviously contained a transient component. The responses in the steady state condition were measured and a mean response amplitude was obtained.

6.1.5 Results

(a) Cochlear Nucleus

The recovery curves of two CN placements are shown in Figures 6-1 and 6-3. The main effects of barbiturate were to depress the recovery of responsiveness compared with the awake animal. There is about 90 percent recovery at an interval of 500 msecs. The other placements showed similar recovery curves. Figures 6-2 and 6-4 show evoked potentials from two of the cats. It can be seen that the amplitude of responses at short intervals is depressed under barbiturate.

(b) Inferior Colliculus

The results obtained at the IC were quite complex and a little surprising. Five placements showed a depression of responsiveness due to barbiturate and a recovery of about 90 percent at an interval of 500 msecs. At one placement, however, responses were depressed under barbiturate for short intervals, but the awake record indicated a facilitation to about 110 percent at an interval of 100 msecs (Figures 6-1 and 6-2). In terms of the model being put forward, this would mean a facilitation instead of an "habituation" decrement. A close look at the histology of this animal suggested that the electrode might be recording near the border of the nucleus and the lateral lemniscus. This issue will be examined further in a later section.

(c) Medial Geniculate Body

The curves obtained at the MG were in keeping with the earlier barbiturate experiments (Figures 6-1 and 6-3). Some placements showed an early barbiturate depression followed by a recovery that exceeded the awake

Figure 6-1

Percentage of control amplitude curves for Cat 2 as a function of stimulus interval for evoked potentials recorded in the IC, CN and MG. Nembutal data are the dotted line and awake data are the solid lines. The middle graph shows facilitation at the IC for the non-Nembutal state.

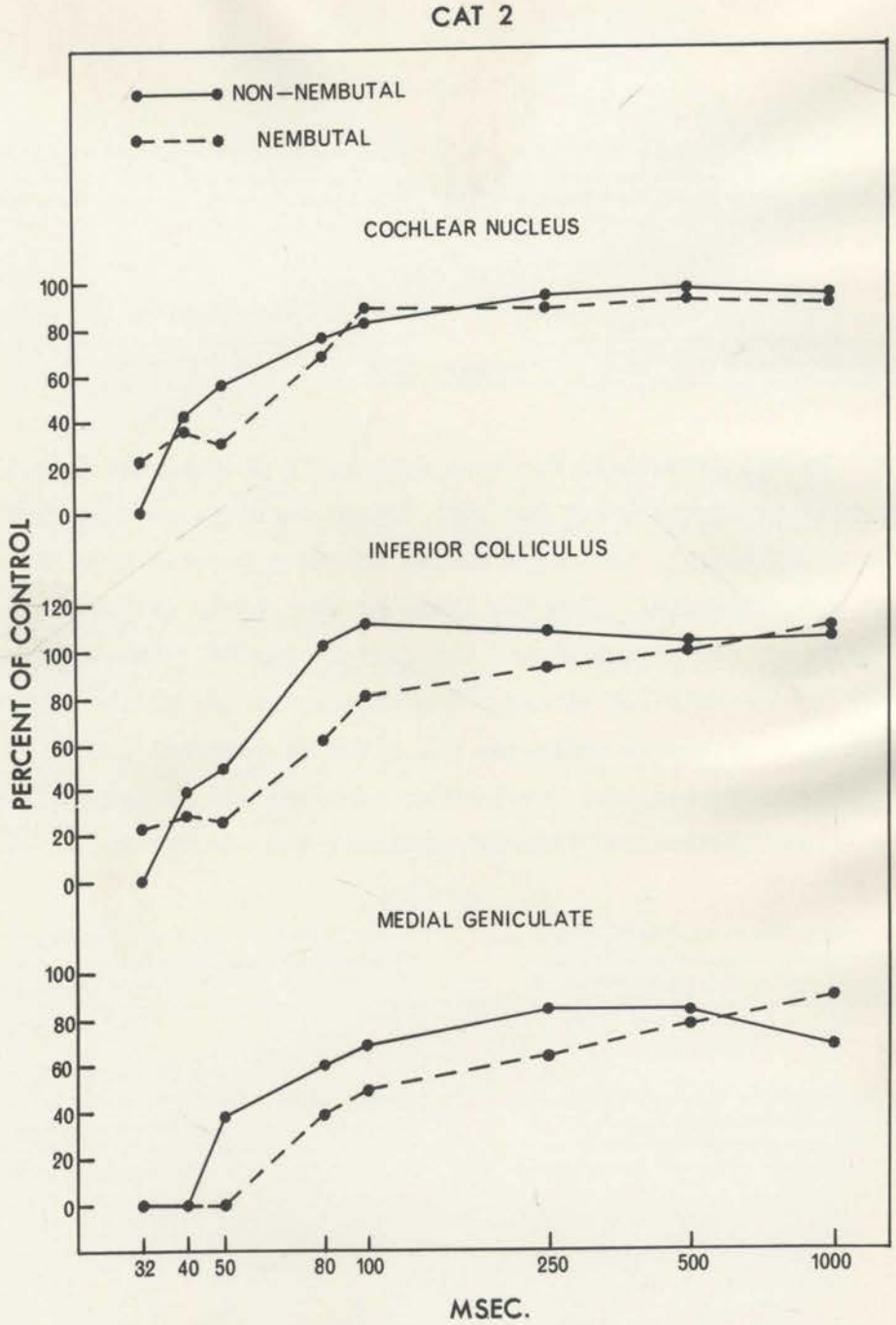

CAT 2
NON—NEMBUTAL
NEMBUTAL
COCHLEAR NUCLEUS
INFERIOR COLLICULUS
MEDIAL GENICULATE
PERCENT OF CONTROL
100
80
60
40
20
0
120
100
80
60
40
20
0
100
80
60
40
20
0
32
40
50
80
100
250
500
1000
MSEC.

Figure 6-2

Evoked potentials recorded from Cat 2 showing the data plotted in Figure 6-1. The left column contains records from the awake animal, the right column contains records from the cat under Nembutal. The top trace of each strip is the MG, the second top is the IC and the next is the CN. The bottom line is the stimulus marker. The figures in the middle of each pair of traces indicates the stimulus interval. The depression in response level under Nembutal can be seen. Amplitude calibration figures represent 100 microvolts.

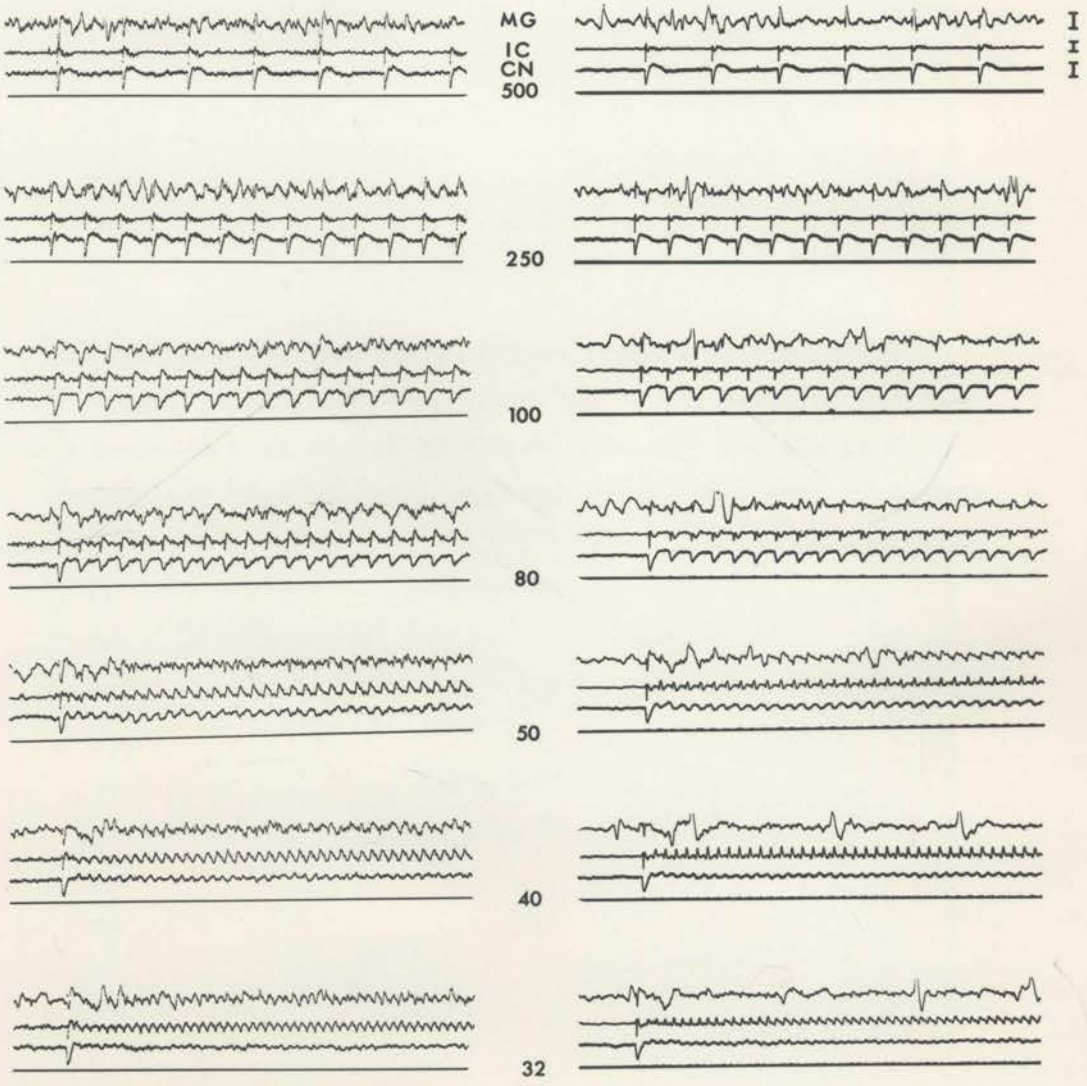

MG
IC
CN
500
250
100
80
50
40
32
I
I
I

Figure 6-3

Percentage of control amplitude curves as a function of stimulus interval for evoked potentials recorded at the CN, IC and MG of one cat (5). The dotted lines are data obtained under Nembutal. The solid lines are data obtained when the cat is awake and alert.

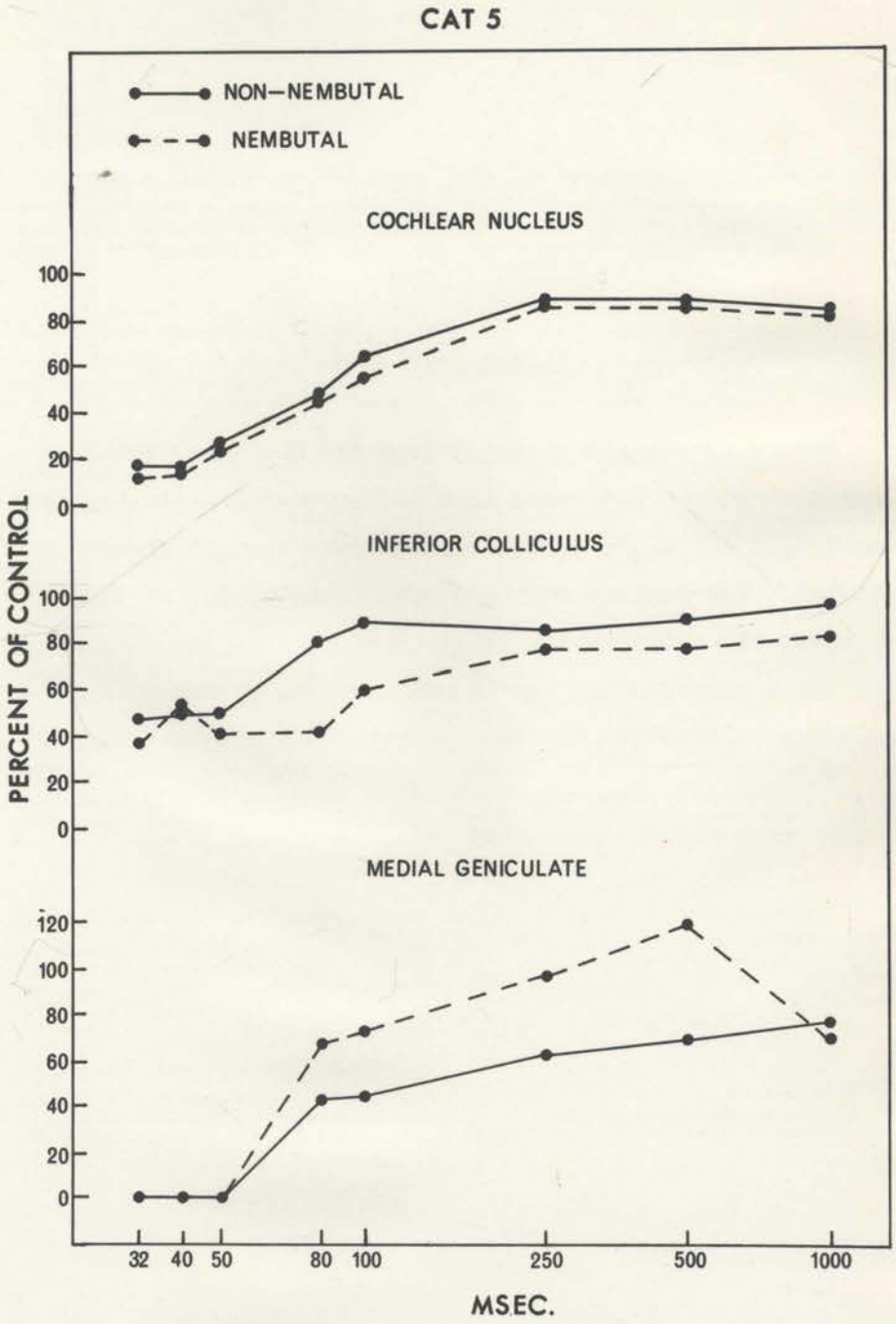
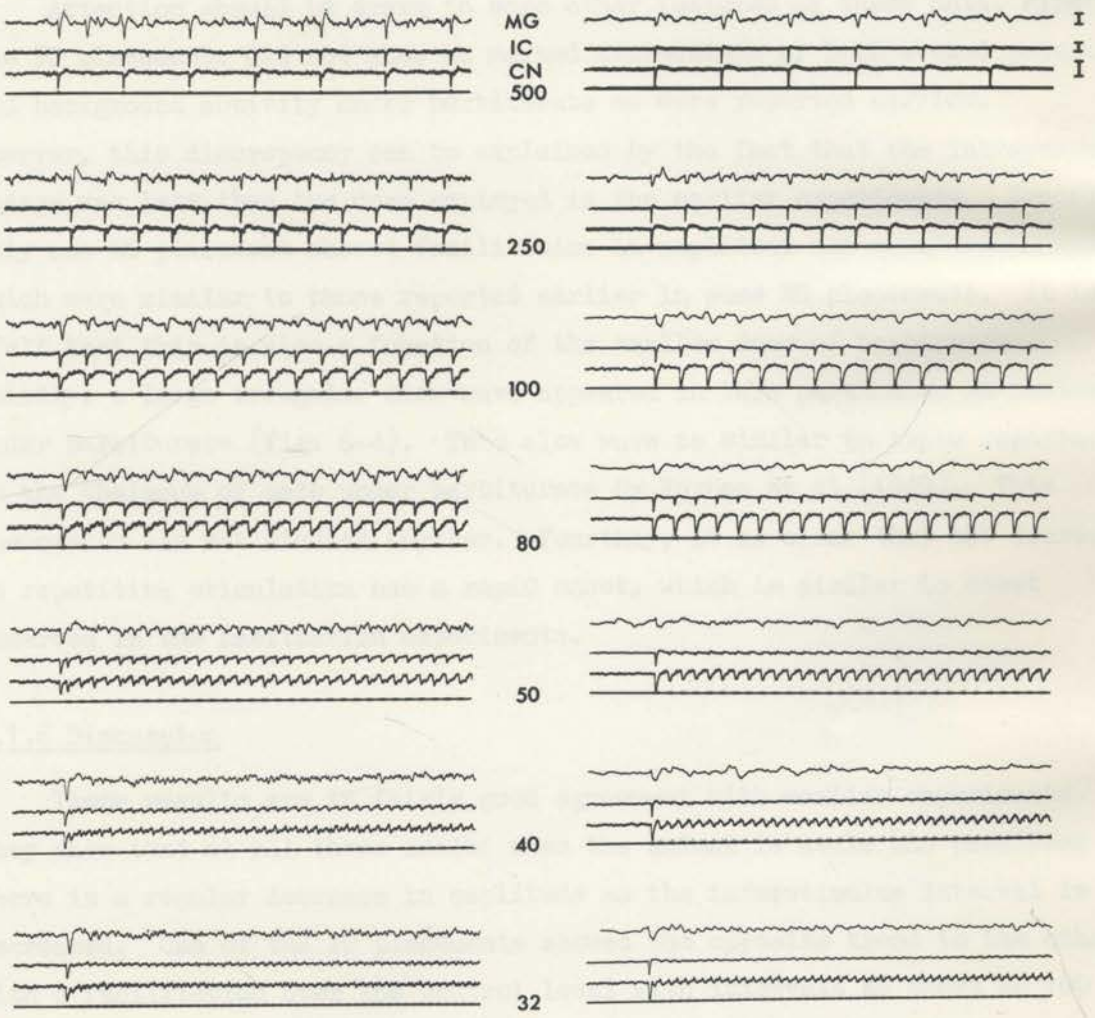

CAT 5
NON—NEMBUTAL
NEMBUTAL
COCHLEAR NUCLEUS
INFERIOR COLLICULUS
MEDIAL GENICULATE
PERCENT OF CONTROL
100
80
60
40
20
0
120
32
40
50
80
100
250
500
1000
MSEC.

Figure 6-4

Evoked potentials recorded from Cat 5 showing the data plotted in Figure 6-3. The left column contains non-Nembutal records and the right column records from the cat under Nembutal. The numbers in the middle of each pair of traces indicates the stimulus interval. Reading from the top, each trace contains MG, IC, CN and stimulus marker. Amplitude calibration figures represent 100 microvolts.


MG
IC
CN
500
250
100
80
50
40
32
I
I
I

state at longer intervals. One placement showed faster recovery under barbiturate conpared with the control, with a facilitation at intervals of 500 - 1000 msecs. This is partly in keeping with the earlier barbiturate experiments where barbiturates often led to an increase in amplitude compared with non-barbiturate levels. Figures 6-2 and 6-4 show evoked potentials recorded under the two conditions for all three nuclei.

Attention should be drawn to some other features of these data. Firstly, the IC placements did not show as marked depressions of both evoked potential and background activity under barbiturate as were reported earlier. However, this discrepancy can be explained by the fact that the intravenous dosage was less than the dose employed in the earlier experiments. Secondly, only one MG placement showed facilitation in amplitude and waveform changes which were similar to those reported earlier in some MG placements. It is felt that this is also a function of the smaller dose of barbiturate. Thirdly, a large irregular slow wave appeared in this particular MG placement under barbiturate (Fig. 6-4). This slow wave is similar to those reported in the thalamus of cats under barbiturate by Forbes et al (1949). This phenomenon was not studied further. Fourthly, it is clear that the decrement to repetitive stimulation has a rapid onset, which is similar to onset observed in the habituation experiments.

6.1.6 Discussion

These results are in fairly good agreement with earlier experiments. They show that at all three nuclei when the animal is awake and paralysed there is a regular decrease in amplitude as the interstimulus interval is decreased. One of the IC placements showed the opposite trend to the others with a facilitation over the control level with intervals as short as 100 msecs. All the CN and IC placements showed a depression in recovery under barbiturate. This would be the expected result according to the theory of intrinsic inhibition. The MG placements showed two patterns. Some placements showed an early depression of recovery under barbiturate with a tendency to facilitation compared with their control at longer intervals. One placement showed quicker recovery under barbiturate and then a facilitation at longer intervals. These results are in reasonable agreement with the earlier experiments and confirm that there can be a facilitative effect which

increases evoked potential amplitude at the MG.

The main interest in these recovery curves is that they indicate a regular function between evoked potential amplitude and stimulus interval with response depression still apparent at 1000 msecs. This would be expected on the basis of the proposed refractory inhibition model of decrements to repetitive stimulation.

While these curves are sufficiently similar to recovery curves reported by Bishop (1964) and Burke and Sefton (1966) to be encouraging, it should be stressed that their technique allowed them to correct for pre-synaptic changes. The curves reported in this experiment are based presumably on both pre- and post-synaptic effects, thus it is difficult to compare the two sets of curves directly.

6.2 Experiment 14 - Amplitude Recovery - II

6.2.1 Rationale and Aim

The results of Experiment 13 have shown that barbiturates depress the recovery curve of evoked potentials recorded in the CN and the IC. In the MG, the results are more complex. There is an initial depression under barbiturate, followed by, in some instances, a facilitation of responding compared with non-barbiturate recovery cycle. The aim of this experiment was to analyse the recovery cycle in the MG using a paired click technique with and without barbiturate.

6.2.2 Subjects, Stimulus Parameters and Procedures

Four subjects implanted for Experiments 5 and 9 were used in this experiment. The stimulus was a 100 db re .0002 microbar, .01 msec click which was delivered through earphones mounted on the animal's head.

The procedure adopted in this experiment was as follows:

(a) Each animal was tested without anaesthetic. He was allowed to settle down in a relaxed position in the test box. This was usually in a small tray containing sawdust. A control record of 50 responses were recorded on the C.A.T. to 50 clicks given at the rate of 1/5 sec. It would have been preferable to have used longer intervals for the control records, but one factor made this difficult. It was felt that the results of Experiment 5 showed that the state of arousal could be an important variable determining evoked potential amplitude in the awake animal. It was found that with intervals longer than five seconds, it was not possible to obtain enough control records from an animal that was alert and awake.

Once the control was obtained, sets of 50 evoked responses were obtained using the paired click technique. Fifty clicks were presented at 1/5 sec but each click was followed by another click at intervals that varied from ten to 200 msecs. The order of interval presentation was selected at random.

It was found necessary to adopt the following control procedures:

(1) At least four minutes were allowed to elapse between each set of

responses obtained. This procedure was designed to eliminate the possibility of one set of stimuli influencing the next set.

(2) Since it was necessary to arouse many animals before some sets of stimuli were given, it was decided to arouse each animal before each set. This procedure seemed to assure an animal that was reasonably alert and awake. However, a number of records were rejected for obvious signs of sleep and were repeated. As a consequence a recording session with an awake animal took many hours. One animal was finally rejected from the experiment because it became impossible to obtain sufficient records in the alert state.

(b) A similar set of records were obtained after the animal was anaesthetised with a surgical dose of barbiturate (45 mg/Kg - Nembutal, Abbott).

6.2.3 Recording System and Data Reduction

The evoked responses were recorded on line with the C.A.T. The memory of the computer was displayed on an X-Y recorder and obtained on a printer which gave a record of each bin number and the digital number stored there. The printer records were analysed in conjunction with the X-Y plots to find the peak-to-peak amplitude of the averaged evoked potential to the second click. This amplitude was then expressed as a percentage of the control record.

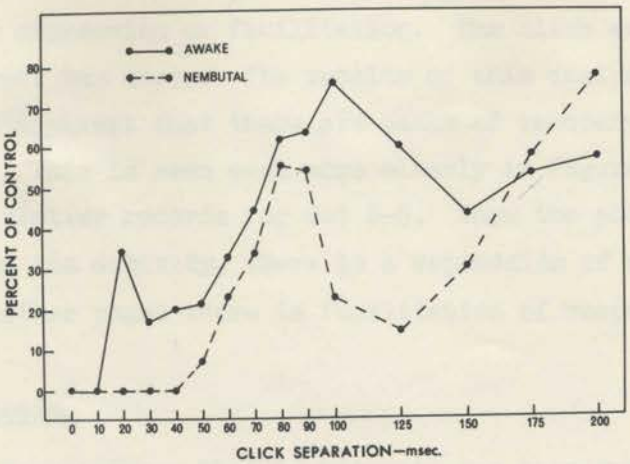
6.2.4 Results

When the percentage of control figures are plotted as a function of click separation (Fig. 6-5), two features are reasonably clear. There is an overall depression of recovery under Nembutal and this is most apparent at click separations up to 40 msec. In two of the animals, S-1 and S-8, there was a tendency for the recovery under barbiturate to be cyclic in nature. This is clearest in the records from cat S-8. With shorter intervals, there is depression below the awake recovery level. Then there is a period when recovery is well above the awake levels. This is followed by another depressed phase and then another period of facilitation. Each animal has at least one period in which the recovery exceeds the awake curve. After these records were taken for this experiment, a further series of observations were carried out on these two animals. Instead of pre-selecting a number of

Figure 6-5

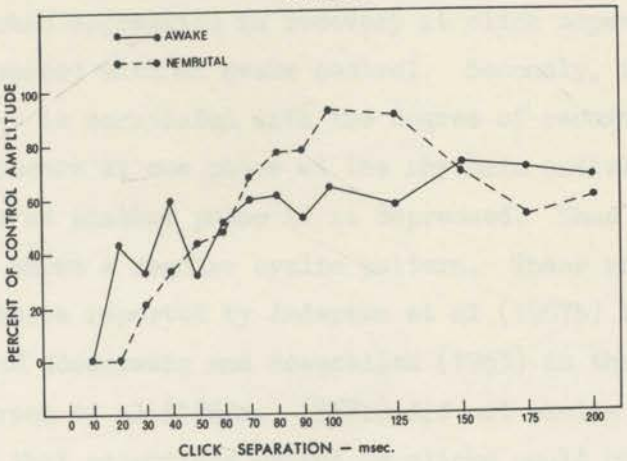
Percentage of control amplitude recovery curves for the second of two paired clicks plotted as a function of click separation. The data from each cat are for the animal under Nembutal (dotted line) and awake (solid line).

CAT S–1 MEDIAL GENICULATE

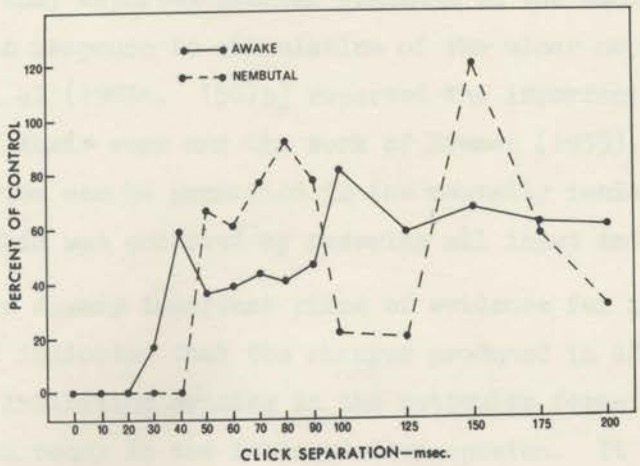


CAT S–4 MEDIAL GENICULATE

PEAK TO PEAK



CAT S–8 MEDIAL GENICULATE



intervals for analysis, the click separation was manipulated until it was observed on the oscilloscope to fall on parts of the rhythmic activity that gave either depression or facilitation. The click separation was then set to the nearest ten msecs. The results of this analysis are seen in Figure 6-6. It is apparent that there are peaks of recovery and troughs of depression. This is seen even more clearly in Figure 6-7, which shows some of the X-Y plotter records for Cat S-8. When the second click is given at one phase of the activity, there is a depression of responding. If it is given at another phase there is facilitation of responding.

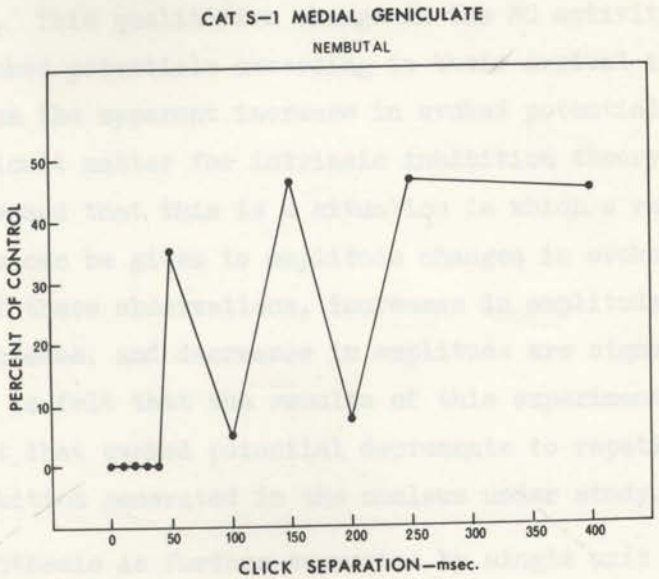
6.2.5 Discussion

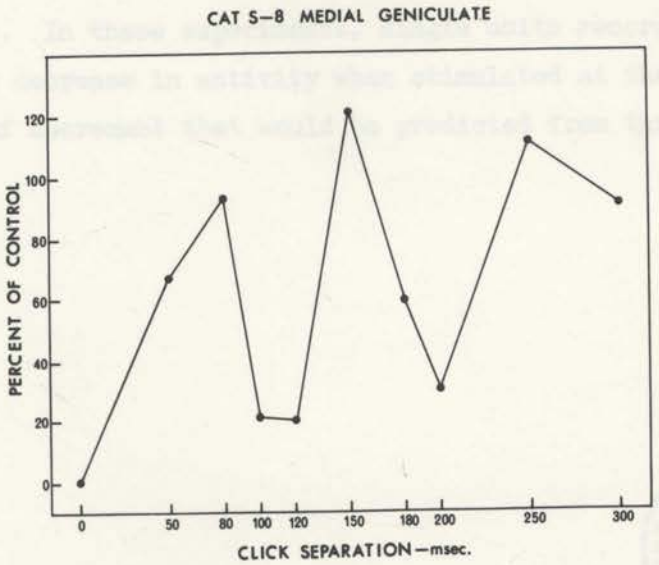
These results show that barbiturate can have two separate effects on the recovery cycle of evoked potentials recorded in the MG. Firstly, it causes a marked depression in recovery at click separation intervals up to 40 msecs compared with an awake control. Secondly, it can produce rhythmic activity that is correlated with the degree of recovery. If the second of two clicks occurs at one phase of the rhythmic activity it is facilitated, if it occurs at another phase it is depressed. Thus the recovery curve under barbiturate shows a regular cyclic pattern. These results are strikingly similar to those reported by Andersen et al (1967b) in the MG of cats and to the results of Rosenzweig and Rosenblith (1963) in the auditory cortex of cats. Andersen et al (1967a, 1967b) did not plot a recovery function, but they showed that potentials evoked by clicks could be either facilitated or depressed depending on the phase of what they call "the thalamo-cortical spindle". They reported similar findings in the nucleus ventralis postero-lateralis in response to stimulation of the ulnar nerve. In addition, Andersen et al (1967a, 1967b) reported the important observation that, as a result of their work and the work of Bremer (1935), it was clear that "the spindle rhythm can be generated in the neurally isolated diencephalon" (p.277). This was achieved by severing all input into the diencephalon.

This is a very important piece of evidence for intrinsic inhibition theory. It indicates that the changes produced in AEPH by barbiturate are not due to inhibition arising in the reticular formation, since this rhythmic activity can occur in the isolated diencephalon. It can be argued, on the basis of experiments reported already in this dissertation on the work of

Figure 6-6

Percentage of control amplitude recovery curves for the second of two paired clicks plotted as a function of click separation. The data from each cat were obtained by adjusting the interval so that depression and facilitation were obtained on an oscilloscope. The marked cyclic recovery can be seen in this graph.


CAT S–1 MEDIAL GENICULATE
NEMBUTAL
PERCENT OF CONTROL
0
10
20
30
40
50
0
50
100
150
200
250
300
350
400
CLICK SEPARATION—msec.

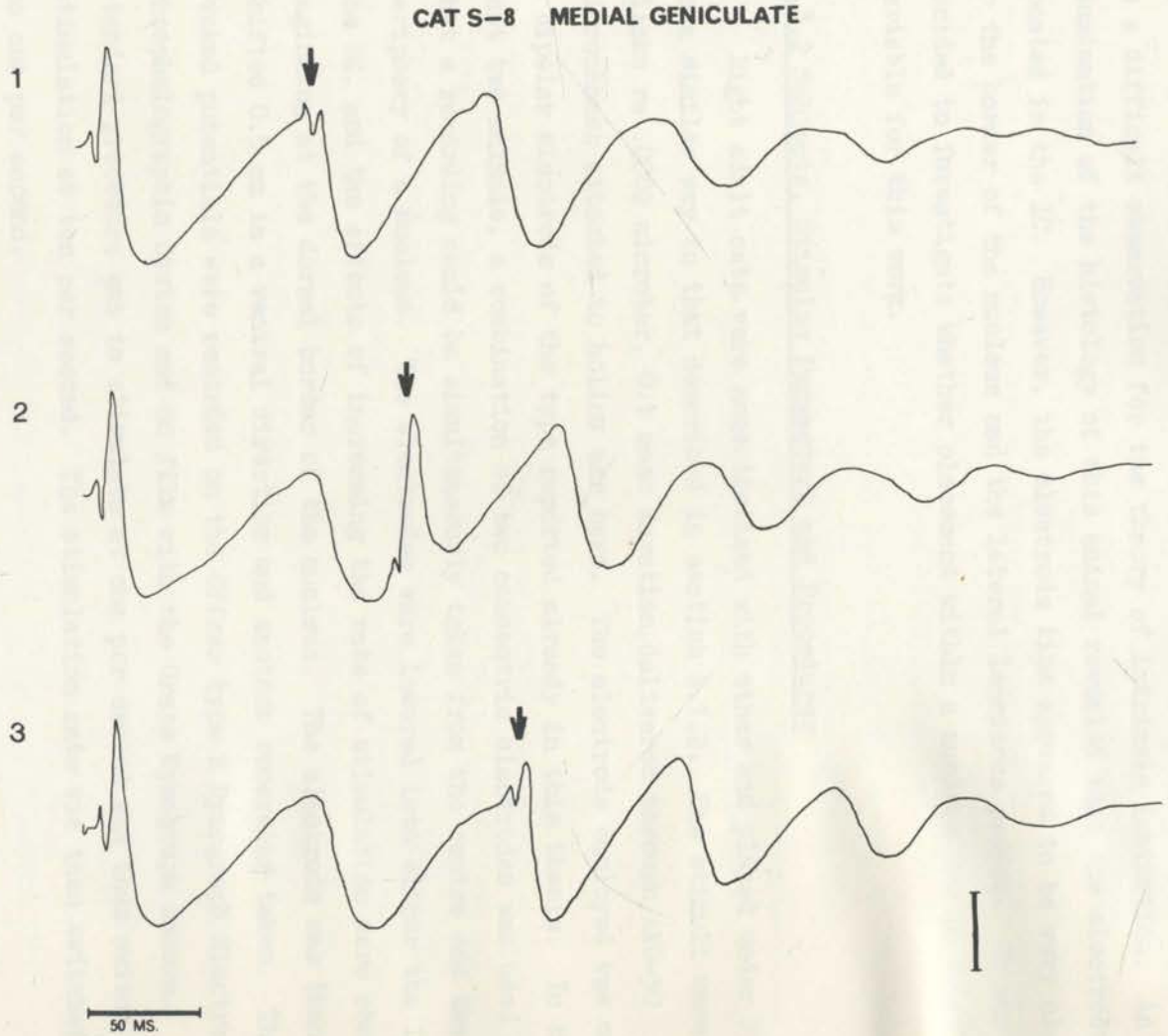

CAT S–8 MEDIAL GENICULATE
PERCENT OF CONTROL
0
20
40
60
80
100
120
0
50
80
100
120
150
180
200
250
300
CLICK SEPARATION—msec.

Andersen and his colleagues, that barbiturates act on the MG in two ways. Firstly, they enhance a depression due to repetitive stimulation, in much the same way as in the CN and the IC. Secondly, they produce an endogenous rhythmic activity in the particular thalamic area being studied (Andersen et al, 1967a). This qualitative change in the MG activity can depress or facilitate evoked potentials according to their arrival in the phase of this activity. Thus the apparent increase in evoked potential amplitude is no longer a difficult matter for intrinsic inhibition theory to explain. It should be stressed that this is a situation in which a reasonable interpretation can be given to amplitude changes in evoked potentials. In the context of these observations, increases in amplitudes are signs of excitation increase, and decreases in amplitude are signs of excitation decrease. It is felt that the results of this experiment give some support to the concept that evoked potential decrements to repetitive stimuli are based on inhibition generated in the nucleus under study.

This hypothesis is further supported by single unit studies of the MG (Aitkin, Dunlop and Webster, 1966; Aitkin and Dunlop, 1968). In the latter study, Aitkin and Dunlop argue that "data in this study support such an hypothesis, at lease for medium rates (1/sec - 10/sec) of stimulation in the MG"(p.58). In these experiments, single units recorded in the MG showed a progressive decrease in activity when stimulated at the above rates. This is the kind of decrement that would be predicted from this hypothesis.

Figure 6-7

X-Y plotter records from Cat S-8. The arrow indicates the evoked potential to the second of two clicks. The first record shows suppression of the second evoked potential (1), the middle record shows facilitation (2) followed by depression again (3). Amplitude calibration figure represents 100 microvolts.


CAT S–8 MEDIAL GENICULATE
1
2
3
50 MS.

6.3 Experiment 14 - Localisation of Electrode Studies

6.3.1 Rationale and Aim

In Experiment 12, it was observed that one of the IC placements showed facilitative effects to medium rates of stimulation (1/sec - 10/sec). This is a difficult observation for the theory of intrinsic inhibition. An examination of the histology of this animal revealed that the electrode was located in the IC. However, the electrode tips appeared to be very close to the border of the nucleus and the lateral lemniscus proper. It was thus decided to investigate whether placement within a nucleus was an important variable for this work.

6.3.2 Subjects, Stimulus Parameters and Procedures

Eight adult cats were anaesthetised with ether and placed under Flaxedil in a similar way to that described in section 6.1.2. The stimuli were 85 db clicks re .0002 microbar, 0.1 msec duration, delivered through AKG-50 microphones attached to hollow ear bars. The electrode employed was usually a bipolar electrode of the type reported already in this thesis. In the last two animals, a combination of two concentric electrodes was used such that a recording could be simultaneously taken from the centre and the periphery of a nucleus. The electrodes were lowered into either the IC or the MG, and the effects of increasing the rate of stimulation were observed, beginning at the dorsal border of the nucleus. The electrode was then shifted 0.5 mm in a ventral direction and another recording taken. The evoked potentials were recorded on the Offner type R Dynograph Electro-encephalographic System and on film with the Grass Kymograph camera. The standard procedure was to stimulate at one per second and then switch to stimulation at ten per second. The stimulation rate was then switched back to one per second.

6.3.3 Results

(a) Medial Geniculate Body

The results for all the MG placements studied show decrements to increases in the rate of stimulation. In every case, increasing the rate of stimulation to ten per second led to a decrease in evoked potential

amplitude. There did not appear to be any differential effects of rate due to recording position within the nucleus.

(b) Inferior Colliculus

The results for the IC placements were often quite different from those of the MG. In some of the nuclei studied, there appeared to be a facilitation when the electrode was leaving the nucleus. This was confirmed in the experiments using the double recording electrode. Simultaneous amplitude depressions and facilitations were obtained (Fig. 6-8). It can be seen in Figure 6-8 that when one electrode is in the centre of the nucleus a decrement in amplitude occurs to stimulation at ten per second. Simultaneously, the other electrode located near the border of the nucleus reveals a facilitation of amplitude when the rate of stimulation was increased. The location of electrodes was identified histologically, and inset into Figure 6-8 are drawings showing the electrode path and the approximate tip locations.

6.3.4 Discussion

This experiment was undertaken to establish whether there were differences in effect of rate of stimulation which depended on the location of the electrode in the IC and MG. No such differences were observed in the MG, but they were observed in the IC. Thus the observation in Experiment 12 of a facilitation to increasing rates of stimulation is not a problem for the theory of intrinsic inhibition.

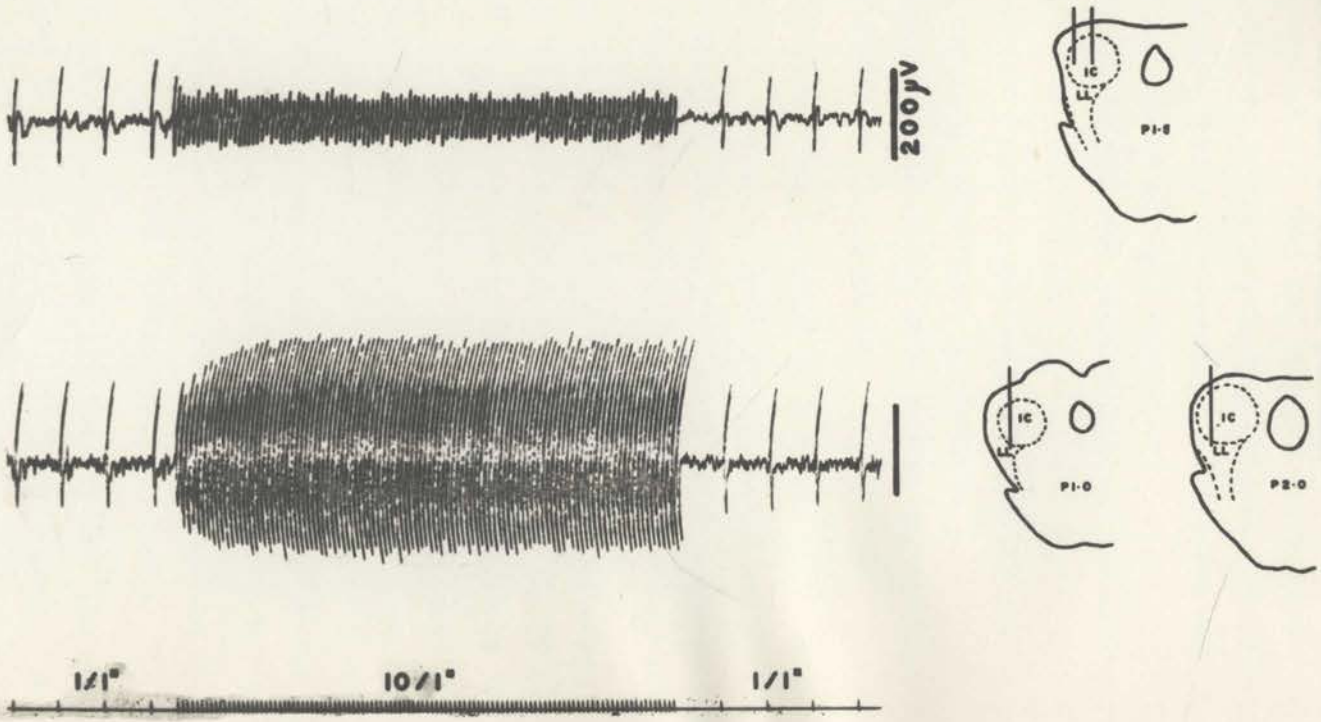
These results also allow a possible explanation of why Marsh et al (1961) obtained evoked potential increments at the IC under AEPH conditions. It might be possible that they were recording from similar areas to those described above. It does not explain why they also obtained increments at the MG, since no increments were observed in this experiment. This does not rule out the possibility that a more extensive search might reveal similar placement effects in the MG.

It is difficult to offer an explanation for the results obtained at the IC. They were usually associated with a change in waveform. Waveform changes have been reported in other nuclei in the auditory pathway (Clarke and Dunlop, 1968). However, there have not been reports of facilitation. It could be

Figure 6-8

An evoked potential recording from the middle of the IC nucleus (top trace); an evoked potential recording from the border of the IC nucleus (middle trace); stimulus event markers (bottom trace). Both records were obtained simultaneously. When the rate of stimulation is changed from 1/1" to 10/1" there is a simultaneous decrease in amplitude (top trace) and an increase in amplitude (middle trace).

Cat I5 0·1 msec Clicks 85 db 1/1", 10/1", 1/1"



speculated that the facilitation is due to a post tetanic potentiation effect at the terminal sites of the lateral lemniscus (Eccles, 1964). This is an interesting issue, but it will not be further pursued.

6.4 Experiment 15 - Dishabituation

6.4.1 Rationale and Aim

It was suggested in section 5.7 that the response decrement to regular stimulation might not constitute a true habituation process, but instead might depend upon an intrinsic inhibitory mechanism. There are now at least three pieces of evidence in support of this suggestion:

(1) The response decrements do not have characteristics that are similar to the characteristics of habituation;

(2) The decrements still occur under barbiturate anaesthesia and in many instances are even enhanced under these conditions;

(3) The recovery of responsiveness in the three nuclei suggest that a long-lasting refractory mechanism is operating.

If this hypothesis is true, then it would be expected that the decrements would not show dishabituation. That is, novel stimuli would not be expected to interrupt intrinsic inhibition, whereas they might be expected to disrupt inhibition arising from polysynaptic areas such as the reticular formation.

Against this argument is the fact that a number of investigators have reported dishabituation by electric shock (Hernández-Peón et al, 1956; A'ltman, 1960). One possible explanation of their results might be the lack of stimulus control. In these studies, a loudspeaker, situated above the animal's head, was used to produce the auditory stimuli. When the animal was shocked and if this led to arousal, then the animal would be likely to stand up in the acoustic field. It has already been seen that if the ear is placed closer to a loudspeaker there is an increase in evoked potential amplitude. Thus this "dishabituation" might be due to an increase in the physical stimulus instead of being a true dishabituation effect.

Another complicating factor in this kind of study is that the middle ear muscles can be activated by shocks and shock-induced movement. Thus a decrement in amplitude would be expected when shocks are paired with the repetitive stimuli. It would seem that there could be two opposing processes brought into play: an increase that is due to acoustic factors and a decrement due to muscular activity.

It was decided to carry out this experiment in three parts as follows:

(1) To study dishabituation using animals with earphones attached to the head,

(2) To study dishabituation in the same animals without earphones and using loudspeakers as the transducers,

(3) To study dishabituation in acute preparations under Flaxedil.

The first two conditions allow some evaluation of the role of change in position in the acoustic field. The third condition allows a test of dishabituation without the influence of the middle ear muscles.

6.4.2 Subjects, Stimulus Parameters and Procedures

Six animals already used in Experiment 5 were used for the first two parts of this experiment. The stimulus was a 105 db "click" of 20 msec duration. The acute preparations were placed under Flaxedil using methods similar to those described in section 5.5.2. The general procedures were as follows:

(1) To each animal (with earphones) electrodes were attached so that shocks could be administered to the chest. An area on the side of each chest was shaved and electrode jelly rubbed onto this area and onto the electrode. The electrodes were attached by rubber straps. A control level of 50 responses to stimuli delivered at 1/10 sec was obtained. Then each animal was stimulated for 30 minutes at the rate of one click every two seconds. At the end of this period shocks were paired with the next 50 auditory stimuli so that they followed each click by 450 msec. The shocks were delivered through a stimulus isolation unit, which was triggered by the waveform and pulse generators controlling the auditory stimulus.

(2) The same procedure was adopted for each animal but without using earphones. The auditory stimuli were delivered through the two loudspeakers in the walls of the test box.

(3) An electrode was placed in the MG of the three acute preparations. Clicks were delivered through the hollow ear bars of the stereotaxic instrument. Shocks were administered by chest electrodes. The stimulus procedures were the same as those above.

6.4.3 Data Reduction

All evoked potentials were recorded on the C.A.T. The memory was displayed on the X-Y plotter and numerical print-out obtained. The peak-to-peak amplitude was calculated from the print-out.

6.4.4 Results

(a) General Comments

It was quickly discovered that the chronic animals were uncomfortable having the electrodes attached to their chest. With four of the animals it was impossible to obtain a satisfactory habituation run. They were always active and moving and they continually endeavoured to remove the electrode straps by scratching or biting. Two of the animals became reasonably quiet and were the only ones tested.

(b) Sessions with Chronic Animals

Since the sample of animals is so small, comments on individual records will be given. The first animal (S-6) showed the following results when tested with earphones:

(1) Evoked potentials recorded in the IC and the MG were reduced following repetitive stimulation (see Fig. 6-9).

(2) The pairing of the shock with the click led to a great deal of gross movement. The size of the evoked potentials was markedly reduced during this period.

(3) When the shock was removed, the amplitude of the potentials increased but only to the habituated level.

(4) A similar pattern was obtained without earphones. The animal moved continually or tried to get at the electrodes on its chest during the presentation of shock.

(5) When the shock was terminated, then the amplitude of the potentials returned to habituation level. At no time was there dishabituation to control levels (Fig. 6-9).

The second animal (S-4) showed a similar pattern at the IC and the MG when tested with earphones. There was much movement with shock, a depression of amplitude and a return to habituation levels. When this animal was tested without earphones a marked change occurred. Now when the animal was shocked

it stood up and remained standing. This produced an increment in amplitude at each nucleus (in fact, the amplitudes became greater than the original control levels).

(c) Sessions with Acute Preparations

Figure 6-10a shows the results for the three MG placements. There is a decrement in amplitude due to repetitive stimulation. The shock produces little change in the evoked potential, either during or after it is removed. The post control of 50 responses to stimulation at 1/10 sec shows a clear return to control levels. It appears that dishabituation does not occur in the MG of animals paralysed with Flaxedil.

6.4.5 Discussion

This experiment is difficult to evaluate since the sample size is so small. The chronic experiments suggest that there are great difficulties in studying dishabituation to noxious novel stimuli. Firstly, the noxious stimulus often leads to gross bodily movements. These movements most likely induce contractions of the middle ear muscles and thus produce decrements in evoked potential amplitude. Such decrements to shock are not apparent in animals under Flaxedil. Secondly, when an animal moves nearer the transducer, there is an apparent "dishabituation". This increment in amplitude is due to variations in the acoustic field. These results suggest that the finding of dishabituation by other workers might be an artefact of change of position in the acoustic field.

The data from the acute preparations indicate that if both muscular movement and position in the acoustic field are controlled, then dishabituation is not observed. Once again, rate of stimulation is an important factor in the observed decrement. These results are in keeping with the theory that the decrements to repetitive stimulation are a refractory process.

Figure 6-9

Averaged evoked potentials for the IC and MG of one cat (S-6). The columns marked E are records taken with earphones, columns marked NE are taken without earphones. The top row shows habituated responses, the middle row shows records taken during the presentation of shock, the bottom row shows post-shock records. This animal did not move during shock presentation and there is no "dishabituation" effect. There is a reduction due to shock.

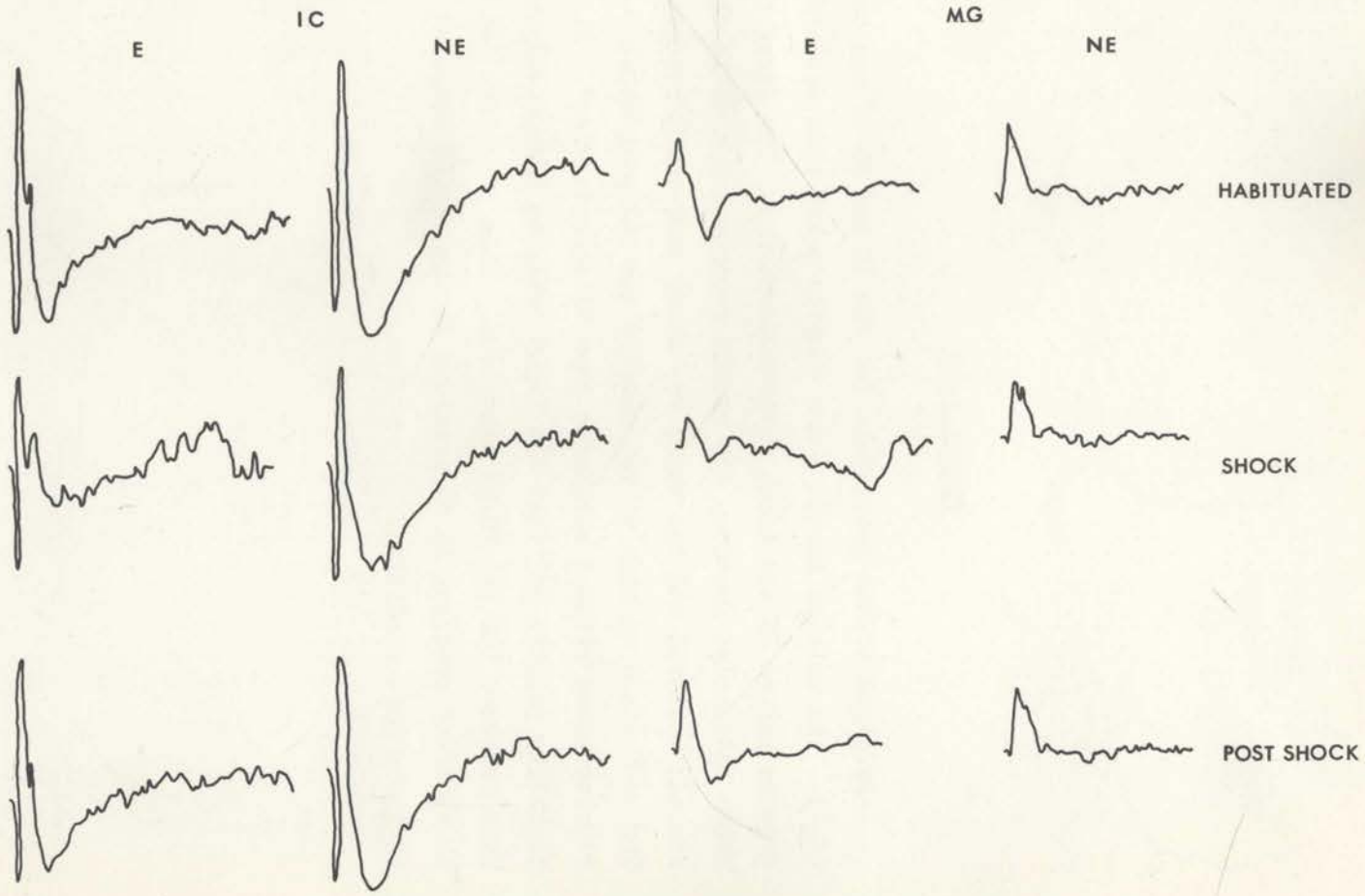

IC
MG
E
NE
E
NE
HABITUATED
SHOCK
POST SHOCK

Figure 6-10

Averaged evoked potentials for the IC and MG of one cat (S-4). The columns marked E are records taken with earphones, columns marked NE are taken without earphones. The first row shows habituated records, the second records taken while shock was administered and the third row shows post-shock records. The cat stood up when it was shocked and the post-shock records taken without earphones show "dishabituation". It should be pointed out that the input leads to the pre-amplifier were reversed for the MG/NE condition. This procedure led to an apparent reversal in MG waveform in the record compared with the MG/E condition.

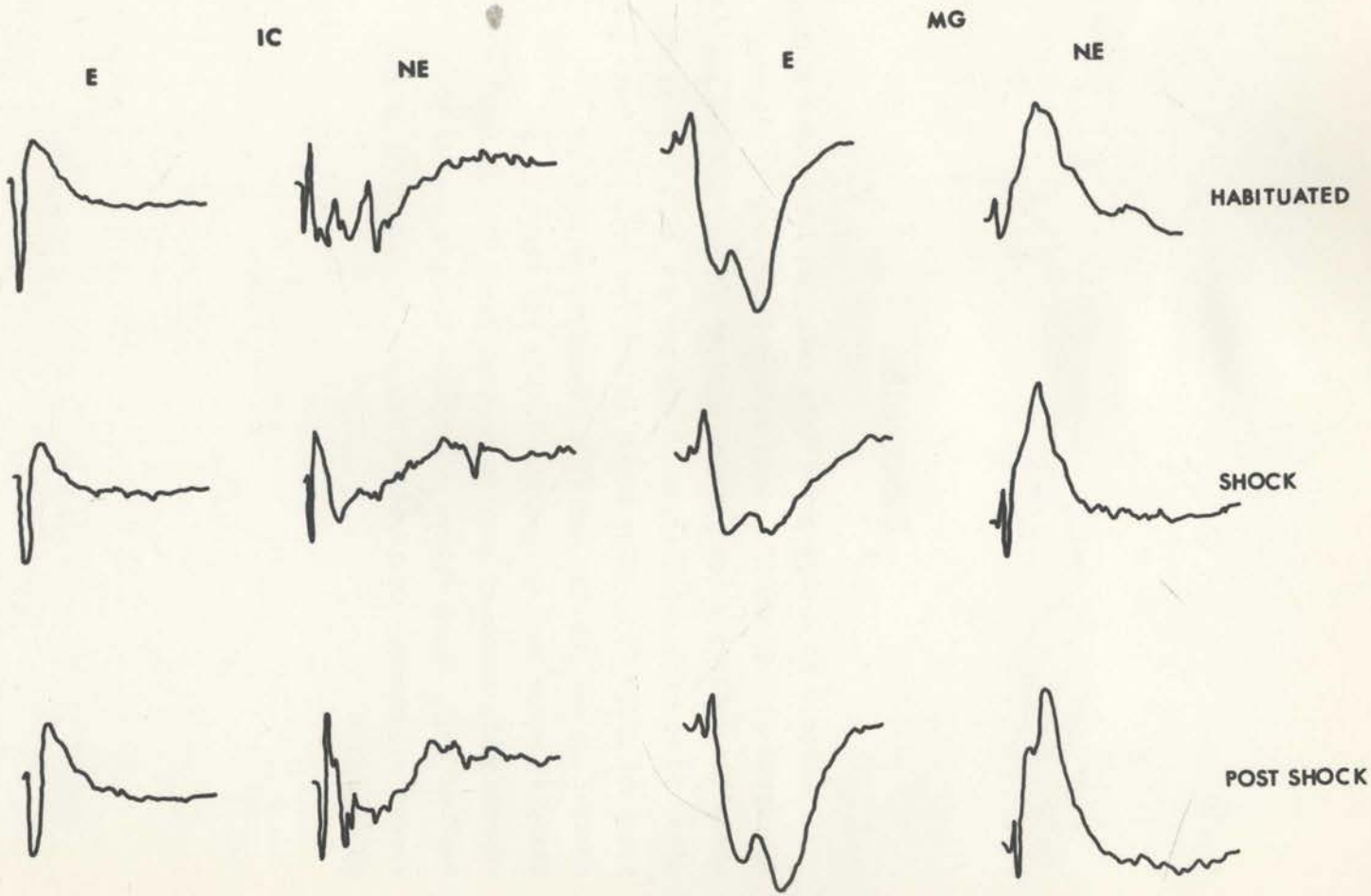
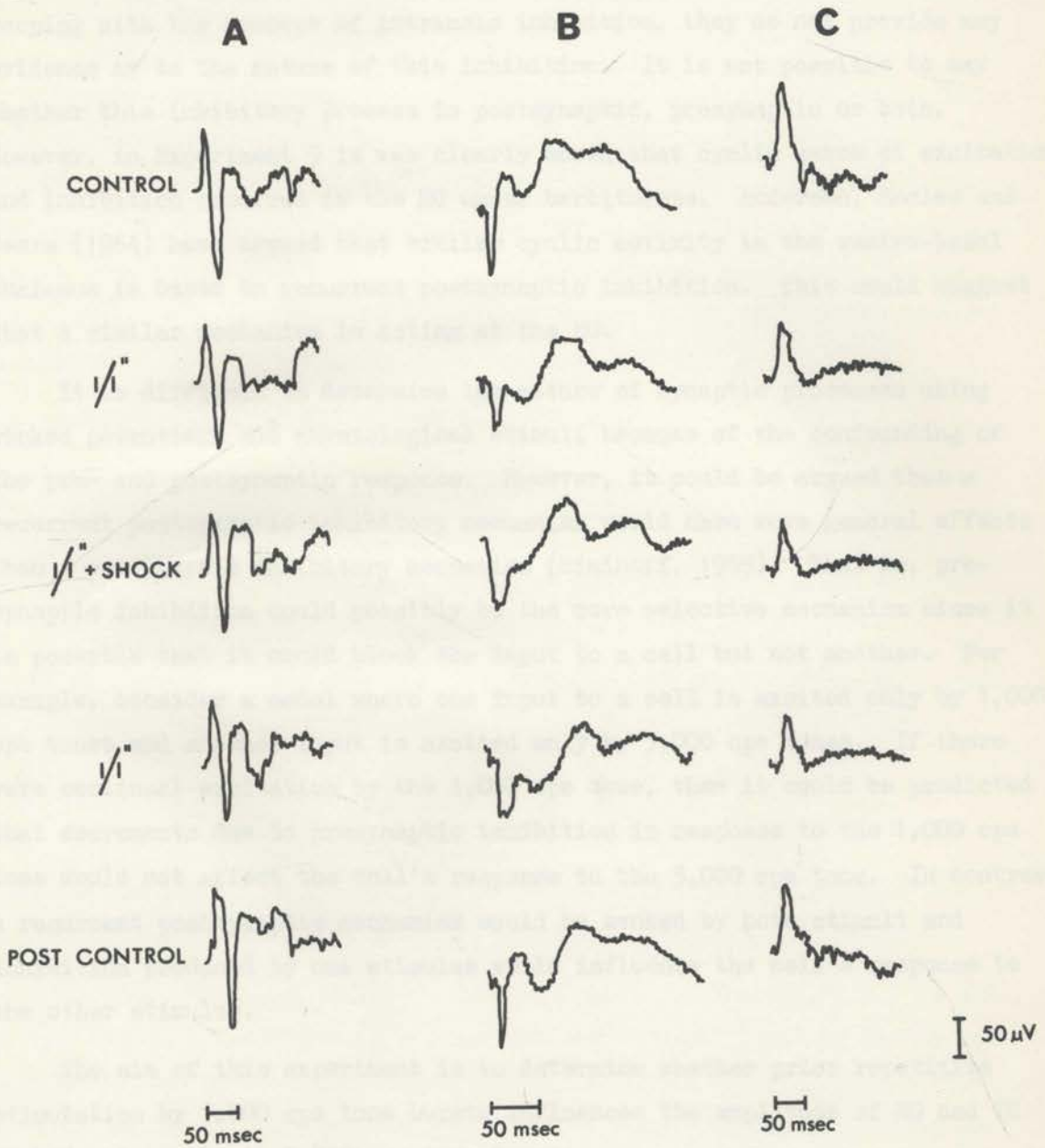

IC
E
NE
MG
E
NE
HABITUATED
SHOCK
POST SHOCK

Figure 6-10a

Averaged MG evoked potentials recorded from three animals paralysed with Flaxedil. Each column shows one MG placement under the following conditions: control at one per ten sec (1), after 30 minutes stimulation at one per sec (2), with paired shock to chest (3), after shock is removed (4), and post-control at one per ten sec (5). There is no sign of dishabituation back to control levels and there is a considerable recovery back to control level during the post-control. Each figure represents the average of 50 evoked responses. Calibration figures represent 50 μV and 50 msec.


A
B
C
CONTROL
1/1"
1/1" + SHOCK
1/1"
POST CONTROL
50 µV
50 msec
50 msec
50 msec

6.5 Experiment 16 - A Test of Intrinsic Inhibition Using Tone Bursts

6.5.1 Rationale and Aim

While the results of the experiments reported in this chapter are in keeping with the concept of intrinsic inhibition, they do not provide any evidence as to the nature of this inhibition. It is not possible to say whether this inhibitory process is postsynaptic, presynaptic or both. However, in Experiment 9 it was clearly shown that cyclic waves of excitation and inhibition occurred in the MG under barbiturate. Andersen, Eccles and Sears (1964) have argued that similar cyclic activity in the ventro-basal thalamus is based on recurrent postsynaptic inhibition. This could suggest that a similar mechanism is acting at the MG.

It is difficult to determine the nature of synaptic processes using evoked potentials and physiological stimuli because of the confounding of the pre- and postsynaptic response. However, it could be argued that a recurrent postsynaptic inhibitory mechanism would have more general effects than a presynaptic inhibitory mechanism (Siminoff, 1965). That is, presynaptic inhibition could possibly be the more selective mechanism since it is possible that it could block one input to a cell but not another. For example, consider a model where one input to a cell is excited only by 1,000 cps tones and another input is excited only by 3,000 cps tones. If there were continual excitation by the 1,000 cps tone, then it could be predicted that decrements due to presynaptic inhibition in response to the 1,000 cps tone would not affect the cell's response to the 3,000 cps tone. In contrast, a recurrent postsynaptic mechanism would be evoked by both stimuli and inhibition produced by one stimulus would influence the cell's response to the other stimulus.

The aim of this experiment is to determine whether prior repetitive stimulation by 1,000 cps tone bursts influences the amplitude of MG and IC evoked potentials to 3,000 cps tone bursts.

6.5.2 Subjects, Stimulus Parameters and Procedures

Five MG placements and five IC placements already used in Experiments 5, 10 and 13 were used. A 1,000 cps, 100 msec tone burst of 80 db re .0002

microbar was used as an "habituating" or "conditioning" stimulus. A 3,000 cps, 100 msec tone burst of 76 db re .0002 microbar was used as a test stimulus. Each tone burst had a rise and fall time of five msec. The intensity difference was due to the differential response of the transducers at each frequency.

The tone was generated by a Bruel and Kjaer beat frequency oscillator and led to a Grason and Stadler Electronic Switch (model 829E). The electronic switch was triggered externally by Tektronix pulse and waveform generators to produce the 100 msec tone bursts with five msec rise and fall times. The stimuli were delivered through earphones mounted on the head of the animal. All responses were averaged on the C.A.T. 400 and the memory displayed on an X-Y plotter and punched out on a printer.

The design of the experiment was as follows:

(1) 50 responses were averaged to 1,000 cps tone bursts given at a rate of 1/10 sec (control 1 Kc).

(2) 50 responses were averaged to 3,000 cps tone bursts given at a rate of 1/10 sec (control A - 3 Kc).

(3) 50 Responses were averaged to 3,000 cps tone bursts given at a rate of 1/1 sec (control B - 3 Kc).

(4) 1,000 cps tone bursts were presented for 30 minutes at 1/1 sec. During this period, 50 responses were averaged every five minutes.

(5) At the end of 30 minutes, 50 responses were averaged to 3,000 cps tone bursts at the rate of 1/1 sec (Ex - 3 Kc).

The critical comparison for this experiment is between Ex - 3 Kc and control B-3 Kc (the control B - 3 Kc record was taken since it has already been shown that a decrement occurs within the first ten responses to clicks given at this rate (section 3.2.5)). If the responses recorded under Ex - 3 Kc conditions were smaller than those recorded under control B - 3 Kc then it could be concluded that the intervening stimulation with 1,000 cps tone bursts had depressed the response to 3,000 tone bursts.

It is unfortunate that there is a difference in intensity between the two tone bursts due to the response of the earphones. It was not possible

to alter both the intensity and frequency settings of the beat frequency oscillator and have no interruption in the stimulus sequence. This means that when the stimulus is changed, there is both a frequency and intensity change. The employment of both a pre- and post- condition controlled for these factors, but it would have been preferable to have had only a frequency change.

To control for arousal state, the control B - 3 Kc and the Ex - 3 Kc were taken after the animal had been aroused. The animals were usually alert but not moving when the records were taken.

Some preliminary work had indicated that it was possible to obtain an on and an off response to tone bursts in the IC and MG. It was desired to use a rise and delay time which would not produce a synchronous response in the periphery of the auditory system. A cat was implanted in the CN and the evoked potential was studied as a function of the rise and delay time of a 100 msec tone burst. Figure 6-12 shows that when the rise time is 1.0 msec or faster, then a marked on and off response appears. It was decided to use a rise and delay time of five msec to control for peripheral synchrony of firing.

6.5.3 Data Reduction and Statistical Analysis

The peak-to-peak amplitude of the first response was determined from the C.A.T. printer records. A t test was carried out on the amplitude differences between control B - 3 Kc and Ex - 3 Kc for both the IC and the MG.

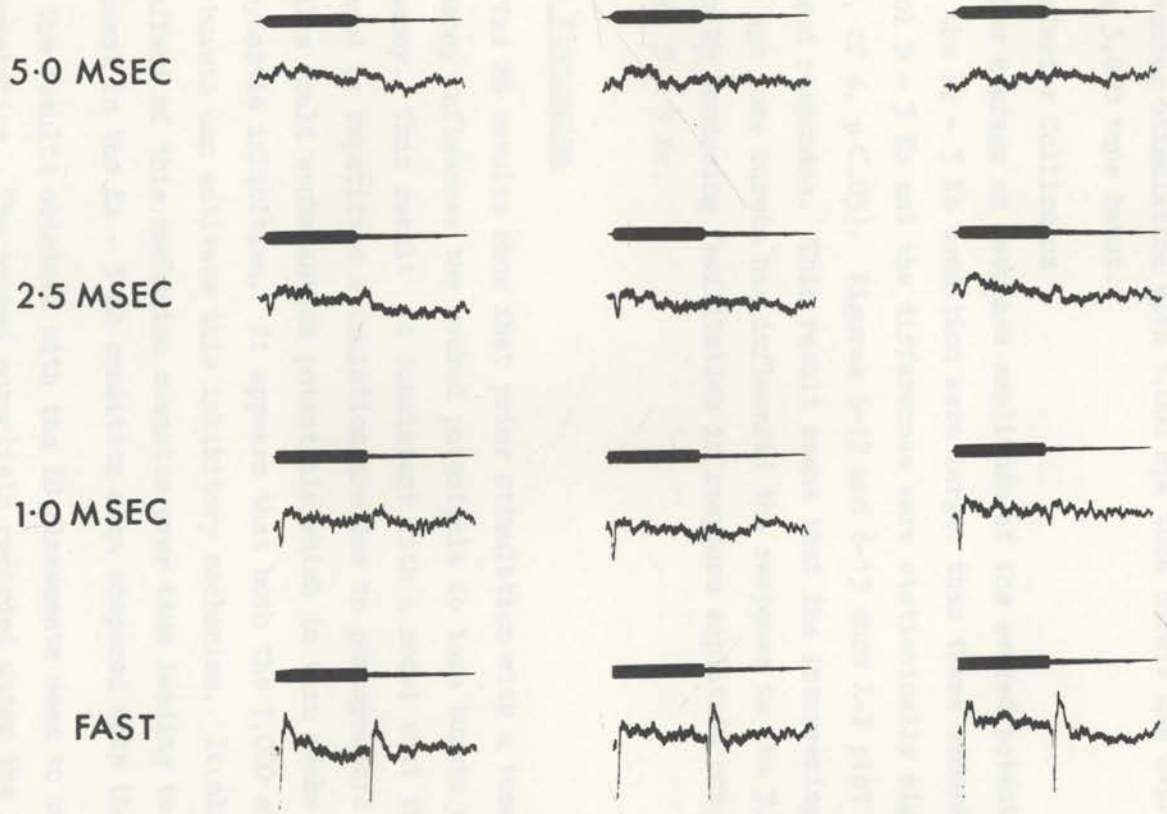
6.5.4 Results

(a) Nature of Evoked Activity

It was soon apparent that, despite the use of a rise time of five msecs, there was a large off and on response in both the IC and the MG. This observation is unusual, since a similar rise time did not produce an off and on response in the CN. It is possible that these off responses are more akin to a removal of inhibition in contrast to the off responses observed at the CN. It should be pointed out that the off responses observed in the CN were very similar to the on response in waveform and amplitude (Fig.6-11). In contrast, the off responses in the IC and MG were quite different from the on response in waveform and amplitude (Figs. 6-12 and 6-13).

Figure 6-11

Oscilloscope photographs showing changes in CN evoked potentials with variation of the rise time of 100 msec tone bursts of 1,000 cps and 80 db re .0002 microbar. The top trace of each photograph shows the stimulus and the bottom trace the evoked potential. Well-defined on and off responses are observed when the electronic switch is set on the first rise time position.


5·0 MSEC
2·5 MSEC
1·0 MSEC
FAST
I 50 μV

(b) Medial Geniculate

The average on response amplitude of evoked potentials recorded under the Ex - 3 Kc were smaller than those recorded under the control B - 3 Kc condition ($t = 3.882$, df 4, $p < .01$). Figures 6-12 and 6-13 show a clear amplitude reduction in the averaged evoked potentials. This means that the intervening stimulation with 1,000 cps tone bursts has depressed the response to the 3,000 tone burst.

(c) Inferior Colliculus

The average on response amplitude of the evoked potentials recorded under the Ex - 3 Kc condition were larger than those recorded under the control B - 3 Kc and the differences were statistically significant ($t = 2.826$, df 4, $p < .05$). Figures 6-12 and 6-13 show X-Y plot records of the averaged responses. This result means that the intervening stimulation by 1,000 cps tone bursts has influenced the response to the 3,000 cps tone bursts by producing facilitation in response amplitude compared with the control B - 3 Kc.

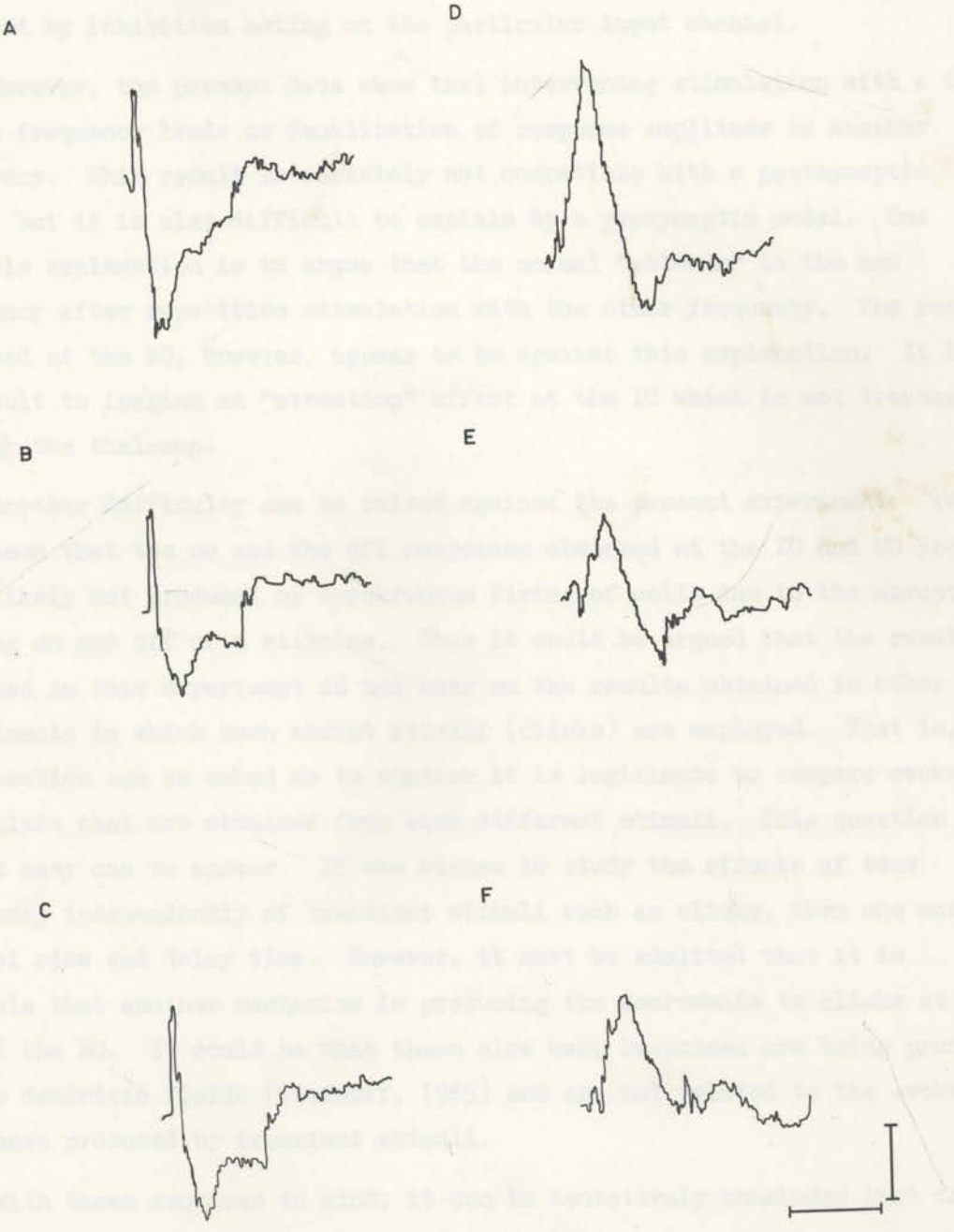
6.5.5 Discussion

The MG results show that prior stimulation with a tone burst of one frequency influences the evoked potentials to tone bursts of another frequency. This result is consistent with a model that the decrements produced by repetitive stimulation are due to postsynaptic inhibition. Each stimulus could evoke action potentials which in turn evoke recurrent postsynaptic inhibition. It appears that both the 1,000 and the 3,000 cps tone bursts can activate this inhibitory mechanism. It also appears that the effect of this mechanism summates over time leading to a greater decrement in the Ex - 3 Kc condition when compared with the control condition.

The results obtained with the IC placements seem to require a different interpretation. The evoked potentials recorded under the Ex - 3 Kc conditions are larger than the control. That is, the intervening stimulation with 1,000 cps tone bursts has influenced the subsequent responses to 3,000 cps tone bursts. This result suggests that a mechanism operating at the IC is different to the one operating at the MG. It is tempting to postulate that the decrements at the IC to repetitive stimulation are based on presynaptic

Figure 6-12

(A) Averaged evoked potential recorded at the IC to 3 Kc tone bursts given at 1/10 sec; (B) averaged IC evoked potential to 3 Kc tone bursts given at 1/1 sec; (C) averaged IC evoked potential to 3 Kc tone bursts recorded after 30 minutes stimulation at 1/1 sec; (D) averaged MG evoked potential to 3 Kc tone bursts given at 1/10 sec; (E) averaged MG evoked potential to 3 Kc tone bursts given at 1/1 sec; (F) averaged MG evoked potential to 3 Kc tone bursts recorded after 30 minutes stimulation at 1/1 sec. Each figure is based on 50 responses. Amplitude calibrations 50 μV, time calibrations 100 msec.


A
D
B
E
C
F

inhibition. The above results are in keeping with a model which postulates that IC cells have one input to one tone burst and another to a tone burst of a different frequency. Any decrements to repetitive stimulation would be produced by inhibition acting on the particular input channel.

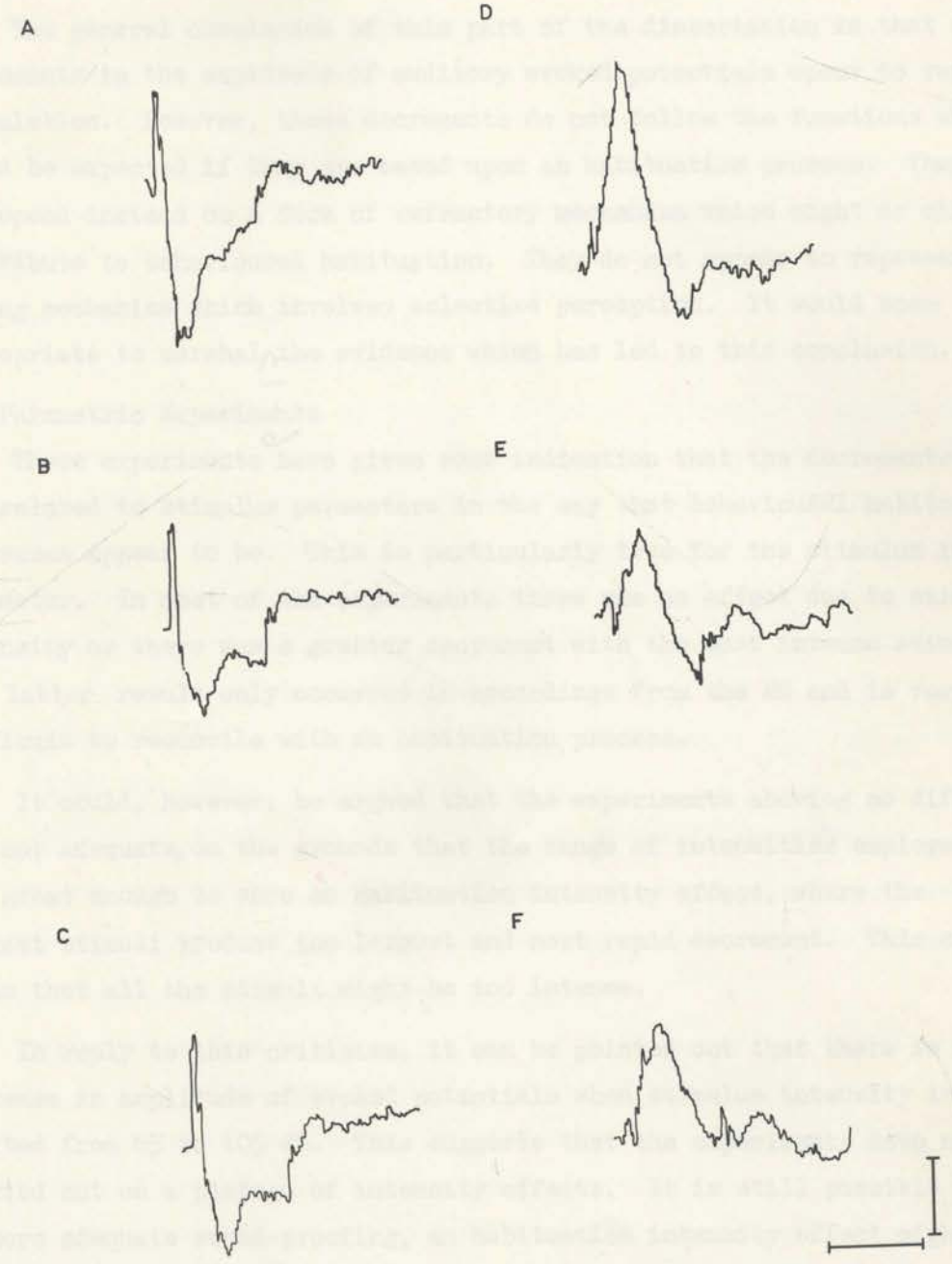
However, the present data show that intervening stimulation with a tone of one frequency leads to facilitation of response amplitude to another frequency. This result is certainly not compatible with a postsynaptic model, but it is also difficult to explain by a presynaptic model. One possible explanation is to argue that the animal "attends" to the new frequency after repetitive stimulation with the other frequency. The results obtained at the MG, however, appear to be against this explanation. It is difficult to imagine an "attention" effect at the IC which is not transmitted through the thalamus.

Another difficulty can be raised against the present experiment. We have seen that the on and the off responses observed at the IC and MG are most likely not produced by synchronous firing of cells due to the abruptly turning on and off of a stimulus. Thus it could be argued that the results obtained in this experiment do not bear on the results obtained in other experiments in which such abrupt stimuli (clicks) are employed. That is, the question can be asked as to whether it is legitimate to compare evoked potentials that are obtained from such different stimuli. This question is not an easy one to answer. If one wishes to study the effects of tone frequency independently of transient stimuli such as clicks, then one must control rise and delay time. However, it must be admitted that it is possible that another mechanism is producing the decrements to clicks at the IC and the MG. It could be that these slow wave responses are being produced in the dendritic fields (Siminoff, 1965) and are not related to the evoked responses produced by transient stimuli.

With these cautions in mind, it can be tentatively concluded that the evoked potential decrements at the MG to repetitive tone bursts appear to be mediated by a mechanism similar to the one proposed for intrinsic inhibition. It would appear that a different mechanism is operating at the level of the IC.

Figure 6-13

(A) Averaged evoked potential recorded at the IC to 3 Kc tone bursts given at 1/10 sec; (B) averaged IC evoked potential to 3 Kc tone bursts given at 1/1 sec; (C) averaged IC evoked potential to 3 Kc tone bursts recorded after 30 minutes stimulation at 1/1 sec; (D) averaged MG evoked potential to 3 Kc tone bursts given at 1/10 sec; (E) averaged MG evoked potential to 3 Kc tone bursts given at 1/1 sec; (F) averaged MG evoked potential to 3 Kc tone bursts recorded after 30 minutes stimulation at 1/1 sec. Each figure is based on 50 responses. Amplitude calibrations 50 μV, time calibrations 100 msec.


A
D
B
E
C
F

6-13

CHAPTER 7

7.0 General Discussion of Habituation Experiments

The general conclusion of this part of the dissertation is that reliable decrements in the amplitude of auditory evoked potentials occur to repetitive stimulation. However, these decrements do not follow the functions which would be expected if they are based upon an habituation process. They appear to depend instead on a form of refractory mechanism which might or might not contribute to behavioural habituation. They do not appear to represent a gating mechanism which involves selective perception. It would seem appropriate to marshal the evidence which has led to this conclusion.

(a) Parametric Experiments

These experiments have given some indication that the decrements are not related to stimulus parameters in the way that behavioural habituation processes appear to be. This is particularly true for the stimulus intensity parameter. In most of the experiments there was no effect due to stimulus intensity or there was a greater decrement with the most intense stimulus. The latter result only occurred in recordings from the MG and is very difficult to reconcile with an habituation process.

It could, however, be argued that the experiments showing no difference are not adequate, on the grounds that the range of intensities employed was not great enough to show an habituation intensity effect, where the weakest stimuli produce the largest and most rapid decrement. This criticism means that all the stimuli might be too intense.

In reply to this criticism, it can be pointed out that there is an increase in amplitude of evoked potentials when stimulus intensity is shifted from 85 to 105 db. This suggests that the experiments have not been carried out on a plateau of intensity effects. It is still possible that, by more adequate sound-proofing, an habituation intensity effect might be achieved using very weak stimuli.

There is another reply to this point, which may be the answer to the objection. Even though the stimuli are very intense, large decrements have occurred to them. That is, perhaps the most important observation is that significant decrements have occurred to intense stimuli. This would

not be predicted for an habituation process. It is not confirmation of a null hypothesis that is important, but the negation of the hypothesis that intense stimuli produce little or no "habituation".

The next finding of the parametric experiments is that there is a marked rate-of-stimulation effect. The most important part of this observation is that no "habituation" occurs to stimuli as slow as 1/20 sec. This indicates that the process recovers fairly rapidly, which can be seen in Experiment 5, where marked recovery occurs when a post control is employed. All of these data are compatible with a localised inhibitory effect.

(b) Experiments Testing Theories of AEPH

(i) The Role of the Middle Ear Muscles

The evidence from the experiments reported in Chapter 5 appear to rule out the possibility that the decrements are produced by the action of the middle ear muscles. There appear to be three pieces of evidence that are against any role for these muscles:

(1) The decrements are not produced by the response of these muscles being conditioned to the interval between stimuli, since the decrements occur to stimuli with random intervals.

(2) Decrements still occur under doses of barbiturate that are known to block the action of these muscles.

(3) Decrements occur when the animals are paralysed by Flaxedil.

It can be concluded that the decrements observed in these experiments are not due to either phasic or tonic involvment of the middle ear muscles.

(ii) The Role of the Reticular Formation

The evidence from Experiments 7, 8 and 9 appears to preclude the possibility that decrements in the amplitude of evoked potentials recorded in the CN, IC and MG are due to inhibition arising in the reticular formation. Barbiturates do not abolish the decrements at the CN and IC; in fact, they tend to enhance the decrement. Decrements under barbiturate are still observed to be a function of the rate of stimulation. This suggests that the mechanism determining the rate effect is not related to the reticular formation, since there is a rate effect both with and without barbiturate.

The effects of barbiturate at the MG are more complex. The evidence of Experiments 7, 8 and 9, in conjunction with Experiments 13, 14 and 15, indicates

that the changes produced by barbiturate are not related to the reticular formation. The following points summarise the evidence:

(1) Experiments 7, 8 and 9 indicate that barbiturates lead to an enhancement of evoked potential amplitude and a change in waveform.

(2) Experiment 14 indicates that the enhancement and change in waveform at the MG are related to a cyclic activity induced in the nucleus by the barbiturate. That is, paired click observations reveal an enhancement in one place of this cyclic activity and a depression in another. This form of cyclic activity has been observed in the isolated thalamus and is thus not produced by the reticular formation (Andersen et al, 1967a).

(3) The failure to obtain dishabituation at the MG is not compatible with reticular formation inhibition (Experiment 15).

The conclusion to be drawn from these experiments is that decrements in amplitude of evoked responses to repetitive stimuli are not produced by inhibition arising in the reticular formation. This evidence does not show, however, that the reticular formation has no effect on these synaptic areas. It is possible that investigations of unit potentials and multi-unit responses would reveal subtle interactions with the reticular formation.

(iii) The Role of the Temporo-Insular Cortex

The hypothesis that the decrements are due to inhibition produced from this area of the cortex has not been tested directly. However, the following difficulties exist for this theory:

(a) The effects of barbiturate on the evoked potentials are a difficult observation for the theory, in the sense that barbiturates are known to affect cortical association areas such as the temporo-insular cortex (Thompson, 1967). It would be difficult to imagine barbiturates enhancing the inhibitory activity of this region.

(b) The effects of changing rate of stimulation and the lack of dishabituation are also difficult for the theory to explain, since Desmedt (1960) has predicted that this cortical inhibition would be abolished by either a change in stimulus parameters or the introduction of a novel stimulus.

This evidence is not a refutation of the theory, but it does render it less convincing.

(iv) The Role of Learning

The hypothesis that the decrements are produced by learning would seem to be invalidated by the following observations:

(a) Decrements still occur under barbiturate at the IC and CN.

(b) The amount of the decrement is a direct function of the rate of stimulation and varies with a change in this rate; it is difficult to see how learning would produce such changes.

(c) Fairly rapid recovery of the responses occurs when stimulation is removed.

(v) Inhibitory Processes Intrinsic to the Auditory Areas

This theory explains the decrements as being due to inhibition that arises in the area from which recordings are taken. It is implicit in the theory that the decrements are not produced by an habituation mechanism. Although the evidence for intrinsic inhibition is also indirect, support for it is provided by the following findings:

(a) Decrements are a function of rate of stimulation, and no decrements are observed with rates as slow as 1/20 sec.

(b) Barbiturates enhance decrements at the CN and IC, and depress recovery in paired click studies.

(c) No dishabituation occurs to novel stimuli and to changes in rate of stimulation.

(d) There is a continuous function relating amplitude decrement with paired click interval.

It should be stressed that there is no direct evidence for an inhibitory process underlying the decrements. Such direct evidence can never be obtained from evoked potential studies. However, of the theories which have been proposed, the intrinsic inhibition theory is the only one which can account for all the relevant findings. Every other theory encounters several pieces of evidence which it is unable to explain.

7.1 The Nature of the Proposed Inhibitory Process

The tentative theory being put forward is that decrements in evoked responses are based on an inhibitory process which is intrinsic to the

particular area from which records are taken. As pointed out in section 4.5, it is assumed that each stimulus evokes a field potential which terminates in a long-lasting period (order of seconds) of depressed excitability. A subsequent stimulus, if it occurs within this period, will evoke a field potential with reduced amplitude. If it is assumed that the process is cumulative then the amount of depression will increase with increased rate of stimulation. The nature of the inhibition is difficult to specify with the evoked potential technique. It could be based on postsynaptic inhibition, on presynaptic inhibition or on both.

The term "refractory" has been used in this dissertation to describe these decrements. It would be more accurate to follow Marshall (1941) and use the term "unresponsive". That is, the amplitude decrements appear to be an indicator of excitation loss, but they need not necessarily indicate a reduction in the number of cells firing. The field potential could simply reflect a decrease in the slow potential associated with the dendritic field (Siminoff, 1965). It is possible that the field potential changes could take place without decrements in unit firing, if the unit firing depended largely on axo-somatic synapses. However, the evidence of Aitkin, Dunlop and Webster (1966) and Aitkin and Dunlop (1968) indicates that unit firing at the MG is depressed at stimulation rates of one per second compared with rates of one per ten seconds. There is no available evidence for the CN and IC, but the above evidence could suggest that synaptic inhibition is involved.

There is some evidence that postsynaptic inhibition is producing the decrements at the MG. Andersen, Eccles and Sears (1964) have put forward evidence which indicates that the cyclic activity observed in the ventro-basal thalamus is due to powerful recurrent postsynaptic inhibition. Since this cyclic activity has been observed at the MG both in the present experiments and in the experiments of Andersen et al (1967a, 1967b), this could suggest that a similar mechanism is acting here. The basis of the decrement at the CN remains to be determined, but it is possible to argue that the results of Experiment 16 indicate that IC decrements are produced by presynaptic inhibition.

There is another possibility that should be considered. It is possible to argue that a small decrement to repetitive stimulation occurs at the CN

(assuming no decrement at the cochlea), and this may be due to post- or presynaptic inhibition. This small decrement is amplified at higher centres because they contain a much larger number of neurones. That is, a decrement in a small number of cells in the CN would lead to a decrement in a larger number of cells in the IC and in turn at the MG.

This amplification hypothesis seems unlikely on two grounds. Firstly, the results of Experiment 4 show that when there is an increase in the CN amplitude due to change in position in the acoustic field, there are no signs of a concurrent increase in either the IC or the MG. This finding suggests that the mechanism for the decrement is not a simple amplification based on the input from the stage before it. Secondly, the differential effects of barbiturates suggest that the processes are intrinsic to the nucleus concerned. For example, barbiturates depress activity in the IC, but this does not appear in the MG records in the way the amplification hypothesis would predict.

Another explanation of the decrements observed to repetitive stimulation is that they are due to decreased synaptic efficacy or low frequency depression. This explanation has been put forward by Thompson and Spencer (1966) to account for habituation of the spinal reflexion reflex in acute cats. However, stimulus-response relationships of the decrements in the auditory pathway are so dissimilar to those obtained by Thompson and Spencer (1966) that it would appear to rule out low frequency depression as mediating in both situations.

Recently, however, Wickelgren (1967 a, 1967 b) has argued that it is not low frequency depression but post-tetanic potentiation (PTP) of inhibitory synapses that is producing habituation in spinal cats. This explanation does appear to account for Wickelgren's results, but some features of these experiments make it difficult to relate the work to that of Thompson and Spencer (1966).

Firstly, although Wickelgren uses the term habituation, she does not attempt to relate the characteristics of her unit habituation to those of BRH. One of the most important aspects of the work of Thompson and Spencer (1966) is that they clearly showed that the decrements in their situation were habituation decrements. In contrast, Wickelgren found that increasing

stimulus intensity led to greater habituation. This finding is not compatible with the work of Thompson and Spencer (1966) and other habituation studies. Secondly, Wickelgren used an unusual stimulus procedure. She presented a burst of 50 stimuli given at the rate of 100/sec. This burst was repeated every ten seconds. The response measured was the number of times the unit fired during the 500 msec burst of pulses. It would seem that this procedure could confound adaptation within the 500 msec burst with a true habituation. In general, Wickelgren's findings are more like those reported in this dissertation.than those reported by Thompson and Spencer (1966). It is probable that she has been studying a process which is not an habituation process. It is not possible at this stage to say whether an explanation based on PTP of inhibitory synapses would account for the data reported in this dissertation.

Another possible explanation of the decrements is that they are related to temporary threshold shifts produced in man by intense impulsive stimuli (Ward, 1962). However, these shifts only occurred with very intense stimuli (145 - 155 db). There does not appear to be evidence of temporary threshold shifts with impulsive stimuli in the range of 75 - 85 db.

7.2 Habituation and the Auditory Pathway

The results of the experiments reported so far indicate that decrements in auditory evoked potentials are not directly related to an habituation process. They may contribute indirectly under certain sets of conditions (for example increasing habituation with increasing rates of stimulation). Moreover, the types of stimulus-response functions associated with this decrement do not appear to be appropriate for a mechanism which will govern selective perception in the non-attending (habituation) situation. For example, the lack of dishabituation by novel stimuli would seem to make this mechanism too inflexible to be the basis for selective habituation.

It should be pointed out that this evidence does not mean that there is no habituation mechanism in the auditory pathway. It does suggest, however, that the majority of cells are not involved. This might appear to be a rather sweeping statement and needs clarification. If the evidence

presented so far is accepted and the decrements observed are refractory in nature, then it is reasonable to argue that there has been an excitation loss in the region of the recording electrodes. Since these electrodes are bipolar and quite large, the loss must involve many neurones. However, it is possible that true habituation decrements are carried by only a small percentage of the neurones being sampled. That is, subtle changes in these cells will be masked by the gross refractory mechanism.

There is some recent evidence that points in this direction. Wall (1967) has reported "novelty detection cells" in the lumbar dorsal horn of the cat. These cells show habituation to intermittent stimuli applied to one region of their receptive field. However, if the stimulus point (touch stimulus) is shifted to another part of the receptive field the response of the cells returns immediately. Furthermore, there were no signs of habituation to intense stimuli. The habituation process was only seen with decerebrate animals and disappeared under barbiturate. The latter evidence is not interpreted in favour of reticular inhibition theory, because Wall notes that barbiturates depress both the spontaneous and the evoked activity of the cells apart from any repetitive stimulation.

Wall, Freeman and Mayor (1968) report similar novelty cells in the dorsal horn of spinal and freely moving rats. Wall (1967) argues that the habituation effects are due to weak presynaptic inhibition that is overcome by intense stimulation. However, be that as it may, his is the first clear-cut evidence for habituation in a sensory pathway. Furthermore, it appears that only a small number of the cells in the area have this selective function.

These studies have important implications for the relationship between habituation and the auditory pathway. They suggest that evoked potentials would most probably obscure the functioning of such a small group of cells, Thus a search for habituation mechanisms in the auditory pathway should concentrate on single unit recordings from awake animals. This recording technique has, as yet, not been consistently achieved in the auditory pathway. It would appear unlikely from Wall's work that such units will be discovered in animals under anaesthetic. This type of experiment may reveal that there is indeed a selective mechanism operating in the auditory pathway.

CHAPTER 8

8.0 Experiments on Attention

8.1 Introduction

Hernández-Peón, Scherrer and Jouvet (1956) reported some findings which have become incorporated into many text books of psychology (Munn, 1961; Grossman, 1967). They reported that the presentation of olfactory or somatic stimuli led to a large reduction in the amplitude of CN evoked potentials to clicks. During the presentation of visual stimuli (two mice in a closed bottle), they reported that, in comparison with control responses, the auditory evoked potentials "were practically abolished as long as the visual stimuli elicited behavioural evidence of attention" (Hernández-Peón et al, 1956, p.331).

The data are reported in two figures, one of which showed the effect of olfactory stimuli on evoked potentials and the other showed the effect of visual stimuli. The latter figure includes photographs of a cat under three conditions. In the first condition, the cat is quiet and relaxed and the sample from the recording of concurrent evoked potentials consists of large well-defined evoked potentials. Under the second condition, the photograph shows the cat directing its visual receptors to look at two rats in a jar (attending). The sample from the recording shows evoked potentials which are reduced to noise level. The third photograph shows the animal once again in a relaxed and quiet condition and the evoked potentials are shown as returning to the same level as in the first condition.

These data are evidence for a marked change in evoked potential amplitude, which the authors have interpreted as indicating a mechanism which blocks or gates afferent information in the peripheral portions of a sensory pathway. The reticular formation of the brain stem is suggested as the site of the sensory inhibition. These data have not gone unchallenged, but before looking at this new evidence, some points should be made about the methods employed in the study.

No indication is given of how many placements were used in the experiment, or of how many placements showed this marked effect. The auditory stimuli

are described as short bursts of rectangular waves at a frequency of 1,000 to 5,000 cps. No evidence is provided as to the exact duration or frequency that is used in the reported data. There was no measurement of stimulus intensity but the stimuli are described as being delivered "at an intensity comfortable to human observers in the same modality" (p.331). This standard of reporting of relevant variables makes it difficult to replicate the study.

However, Jane, Sharpless and Jasper (1962) reported that when cats oriented to either a visual stimulus (a rat in a bottle) or an auditory stimulus (a squeak of a rat), there was a marked increase in amplitude of evoked potentials recorded simultaneously in the visual and the auditory systems. They implanted electrodes in the visual and auditory cortex of five cats, three of which were also implanted in the medial and lateral geniculate. They stimulated with clicks and flashes presented simultaneously at one per sec. They found habituation in both the visual and auditory systems, but a subsequent increase with the "attention" stimuli. These data show changes that are in the opposite direction to that of Hernández-Peón et al (1956).

Jane et al interpreted these changes in terms of improved synchronisation or less dispersion of the responses. They argued that such effects are not selective or indicative of a gating mechanism, since they affected both the visual and auditory systems. That is, a visual stimulus did not lead to a reduction in the auditory responses and vice versa, as would be predicted from the earlier study.

However, this interpretation should be treated with some caution. They "habituated" the responses first and the change could be interpreted as a "dishabituation" effect rather than an "attentive" effect. More importantly, they did not control for the position of the animal in either the acoustic or the visual field, and the evidence from Experiment 15 suggests that the apparent "dishabituation" or "attention" effect could be an artefact of this variable. That is, any effects of attention could have been hidden by changes due to position in the stimulus fields.

Horn (1960) and Horn and Blundell (1959) reported that evoked potentials recorded in the visual cortex of cats to flashes were reduced in amplitude when the animals "looked intently" at a mouse, placed between cat and light

source. The position of the animal's head in the field did not affect the size of the responses over an angle of 75° to the right and left of the light source (a stroboscope). They also controlled for variation in the diameter of the pupil by atropine. These authors have interpreted the data to imply three things: firstly, that "attentive" effects are only obtained if the animal is making visual searching responses; secondly, that Hernández-Peón and his colleagues would argue that increases should occur when the animal "attends" to a stimulus in the same modality as the evoked responses; and thirdly, that the results obtained by Hernández-Peón et al (1956) are probably due to the animal's listening for sounds coming from the rats in the jar. This would make the data simply a function of auditory searching behaviour, although this explanation would in itself need further explanation.

These interpretations can be challenged on several grounds. Firstly, it is possible that the presence of the rat in the visual field blocked part of the effects of the flashes. Secondly, it would seem that Hernández-Peón would predict a decrease in amplitude and not an increase as Horn suggests. For example, it would seem plausible for Hernández-Peón to argue that if an animal directs its receptors toward one visual stimulus (the rat), then one might expect a reduction in the response to the non-attended stimulus (the flash). It would seem that these data can be readily interpreted by Hernández-Peón's theory and in this sense, they are not a critical test of this position.

There appears to be a simpler explanation of the results obtained by Hernández-Peón. We have seen that when an animal moves, then there can be reductions in evoked potential amplitude. It is most likely that these reductions are due to the activation of the middle ear muscles (Carmel and Starr, 1963). It would seem that Hernández-Peón's findings need to be tested by an experiment in which there is some control over the movement of the animal.

8.2 Experiment 17 - The Effects of Attention

8.2.1 Aim

The aim of this experiment is to determine whether reductions in auditory evoked potentials occur when an animal directs his receptors at a stimulus of another modality; and to determine whether these reductions are a result of movement artefacts.

8.2.2 Subjects, Electrode Implantation, Recording Technique

Five animals who were implanted for Experiment 3 were used in this experiment. There were five CN placements, four IC and five MG placements. Evoked potentials were recorded on the Offner EEG system as described in 3.2.4.

8.2.3 Stimulus Parameters

The auditory stimuli were 0.2 msec clicks of 85 db re .0002 microbar. The stimuli were given at the rate of 1/5 sec. This rate was selected as a compromise between possible decrements due to rate of stimulation and the difficulty of obtaining enough responses under any one experimental condition with longer intervals.

8.2.4 Test Environment, Procedures

The test cage was the same as that used for the habituation experiments (section 2.4.2). The stimuli were delivered through the two loudspeakers. To control for movement in the acoustic field, the animal was placed inside another small wire box inside the test box. This cage restricted the range of the animal's movements. (This experiment was carried out before the technique of mounting earphones on the head of the animal had been developed.

Each cat was given time to settle down in the cage and ten responses to clicks given at 1/5 sec were recorded and regarded as the control for the awake but non-attentive state. Then a white mouse was introduced into the test box alongside the cat but outside the small wire box. Auditory stimulation was presented throughout the period in which the mouse was in the cage.

Two observers monitored the behaviour of the cat through the one way glass, while a third noted on the pen recording the behaviour reported by the other two observers. Each cat's behaviour during this period was grouped into two classes, (1) attentive but motionless; (2) attentive plus movement. Attention was defined as directing the visual receptors toward the freely moving mouse.

8.2.5 Data Reduction and Statistical Design

Ten responses at each nucleus were obtained for the three conditions: non-attentive control, attentive but motionless and attentive with movement. These responses were measured by ruler and converted to microvolts.

The data were analysed with an overall analysis of variance and t tests carried out on the difference between the control values and the two conditions of attention. It was predicted that there would be significant differences between control and attentive-movement conditions for each nucleus and no significant difference between control and attentive-motionless conditions for each nucleus. Type I and II error rates of .05 were set.

8.2.6 Results

The results of the statistical analyses are set out in Table 34. At each nucleus, the analyses of variance show that there is a significant treatments effect. The results of the t tests (Table 34) show that at each nucleus there is a significant difference between the control amplitudes and the attentive-movement amplitudes, but that there is no significant difference between the attentive-motionless condition and the control. Figure 8-1 shows the evoked responses from one cat which is typical of the sample. The responses in section A (Fig. 8-1) show evoked potentials recorded at the IC, CN and MG when the cat is relaxed and awake. The responses in section B of the figure show potentials when the cat is motionless and attending to the mouse. There is no change in the amplitudes of the responses. Section C of the figure shows the marked changes in amplitude that occur when the animal is attentive and moving. Section D shows responses recorded when the mouse is removed and the cat is once more relaxed and awake. The response amplitude is restored. It should be noted that the photographs of the cat

TABLE 34

SUMMARY OF STATISTICAL ANALYSES OF EXPERIMENT 17

(a) Cochlear Nucleus

SOURCE	DF	SS	MS	F
Treatments	2	2,005.8	1,002.9	12.15 **
Subjects	4	3,538.0	8,845.0	
Residual	8	667.2	83.4	
TOTAL	14	6,211.0		

t-tests

SOURCE	Mean Amplitude (μV)	df	t
Control	133.2		
Attention	135.4	4	0.94
Attention + Movement	109.6	4	3.04 *

(b) Inferior Colliculus

SOURCE	DF	SS	MS	F
Treatments	2	6,706.6	3,353.3	11.50 **
Subjects	3	22,619.7	7,539.9	
Residual	6	1,751.4	291.9	
TOTAL	11	31,077.7		

t-tests

SOURCE	Mean Amplitude (μV)	df	t
Control	232.3		
Attention	236.8	3	0.66
Attention + Movement	184.5	3	3.39 *

(c) Medial Geniculate

SOURCE	DF	SS	MS	F
Treatments	2	4,442.0	2,221.0	54.8 ***
Subjects	4	31,500.0	7,875.0	
Residual	8	324.0	40.5	
TOTAL	14	36,266.0		

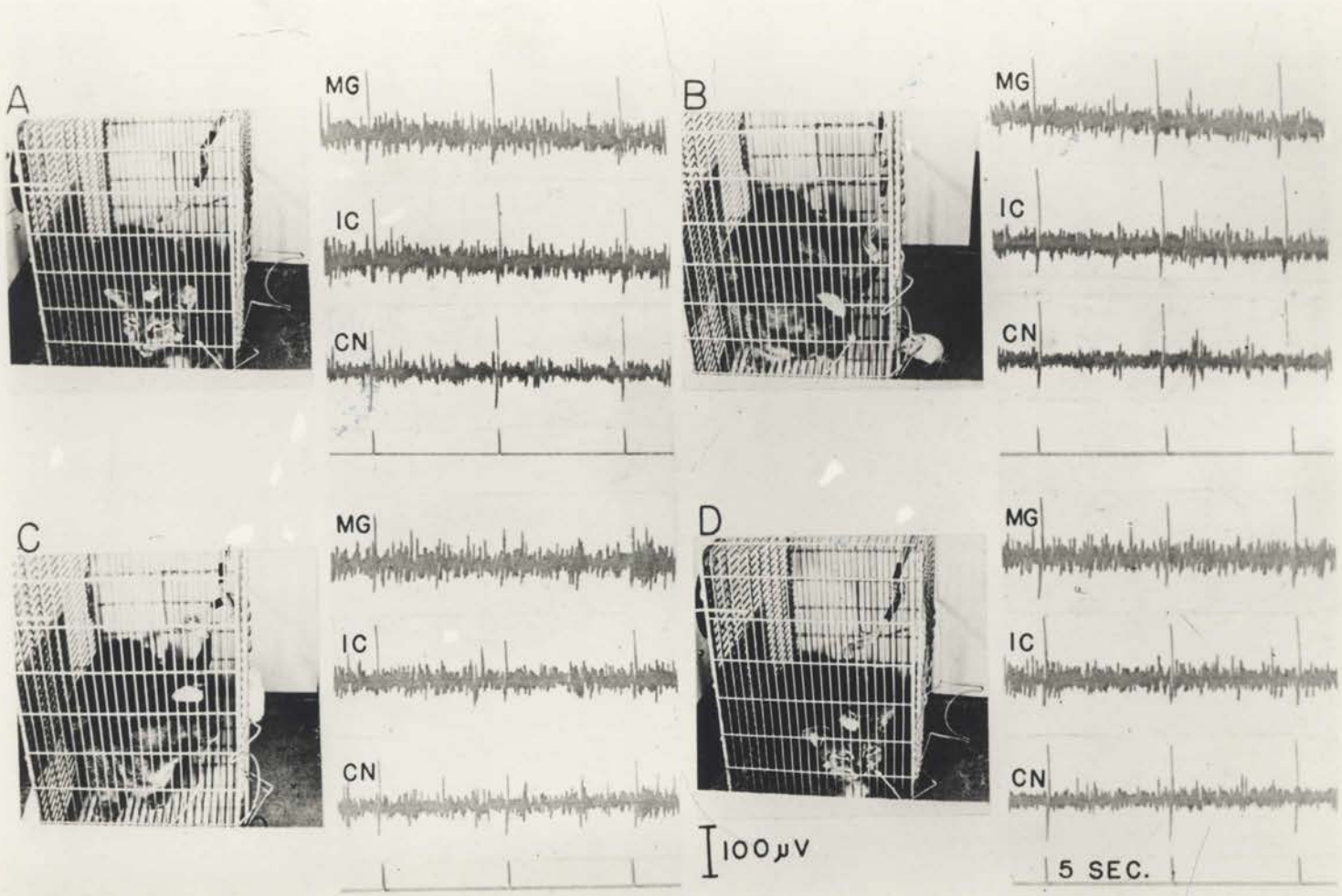
t-tests

SOURCE	Mean Amplitude (μV)	df	t
Control	170.8		
Attention	167.6	4	2.05
Attention + Movement	132.8	4	4.01 *

* significant at .05 level
** significant at .01 level
*** significant at .001 level

Figure 8-1

Evoked potentials recorded while the cat is relaxed and motionless (A); evoked potentials recorded when the cat is attending to a mouse and also motionless (B); evoked potentials recorded while the cat is moving and attending to the mouse (C); evoked potentials recorded when the cat is once again relaxed (D).


A
MG
IC
CN
B
MG
IC
CN
C
MG
IC
CN
D
MG
IC
CN
100μV
5 SEC.

watching the mouse were not taken simultaneously with the records displayed. It was not possible to take photographs of the inside of the main box when it was closed. These photographs were taken later to illustrate the type of movements observed.

8.2.7 Discussion

The hypothesis being tested in this experiment is that changes in evoked response amplitude due to an animal attending would be correlated with movement. The results show that the only changes occurring when the animal is attending (directing its receptors at a visual stimulus) are those correlated with movement. There is no change in response amplitude when the animals are attending and motionless. Similar results occur at each of the three nuclei tested.

These data are consistent with the work of Carmel and Starr (1963), who reported that middle ear muscles in waking cats are readily activated by non-acoustic factors. It could be argued that this "movement" effect is due to intensity variation in the acoustic field. This explanation is felt to be unlikely, since the small inner cage severely restricted the movement of the cat to a range of positions at which there is little variation in evoked response amplitude (Experiment 1).

It is difficult to reconcile these data with the findings reported by Hernández-Peón et al (1956). It should be noted that in section C of Figure 8-1 there are signs of movement artefact on the record. A closer inspection of the records of the evoked potentials in the above study by Hernández-Peón et al (1956) shows that these workers have recorded with very fast paper speeds on their pen recorder. It has been noticed in this laboratory that such fast recording speeds make it very difficult to detect movement artefacts on pen recorders.

It would appear from the results of this experiment that attempts to study attentive behaviour, while recording in the auditory pathway, should control for both movement and position in the acoustic field. The obvious control for movement-induced effects is to sever the middle ear muscles, which was attempted without success during this work. The results of the work were published (Dunlop, Webster and Simons, 1965), but while the paper

was in press, Starr (1964) reported no decrements due to attention in subcortical parts of the auditory system of cats with severed middle ear muscles. His study confirms the results of the present experiment and indicates that decrements previously attributed to attention were, in fact, artefacts of middle ear muscle movements.

8.3 Experiment 18 - Attention Effects in Acute Preparations

8.3.1 Introduction and Aim

Some preliminary observations were carried out on the animals used in Experiment 10 (Flaxedil study) after they had been tested. These observations suggested that painful stimuli(pinching of leg or flank of the animal) produced decrements in evoked potentials recorded at the MG,but did not influence potentials recorded at the CN or IC.

These observations suggested that painful stimuli might be capable of "blocking" or influencing sensory transmission at the level of the thalamus. It was decided to examine whether painful stimuli would produce reductions in the amplitude of evoked potentials recorded at the MG. The results of Experiment 17 indicated that some control over movement would be necessary. It is obviously difficult to obtain such control in chronic animals, so it was decided to use acute preparations paralysed with Flaxedil.

8.3.2 Subjects and Electrode Implantation

Five adult cats were anaesthetised with ether and placed under Flaxedil using methods already described in 6.1.2. The subjects were placed in a shielded box while positioned in a stereotaxic instrument (Trent Wells). Artificial respiration and regular injections of local anaesthetic were given. All placements were verified histologically (6.1.2). Bipolar recording electrodes were implanted.

8.3.3 Stimulus Parameters, Recording Techniques and Procedures

The stimulus employed was an 85 db re .0002 microbar, 20 msec click, which was delivered through AKG-50 earphones attached to hollow ear bars on the stereotaxic instrument.

Evoked potentials were recorded on the C.A.T. and the amplitudes of the averaged response were obtained. Individual evoked responses were recorded by a Grass Kymograph camera mounted on an RM 561 Tektronix oscilloscope.

It was decided to use an air puff directed at the cornea of the eye as a painful stimulus. Since the animal was paralysed (no signs of skeletal

reflexes), the eyelids could not protect the eye from the air puff. The procedure was to record 50 responses to clicks presented at the rate of 1/sec as a pre-control. Then 50 responses were recorded during which time rapid air puffs were directed at the eye. No attempt was made to synchronise the puff and the click in time, although on many occasions there was an overlap. Following this, 50 responses were recorded as a post-control. It was realised that the use of a stimulation rate of one per second would by itself introduce amplitude decrements within the block of 50 responses. However, a longer period of presentation of painful stimuli was not desired, so some confounding with the effects of rate was accepted. It was felt that a pre- and post-control condition controlled for this factor.

In two of the animals concurrent recordings were taken at the IC, and in one cat recordings were also taken at the CN, in an attempt to rule out peripheral effects due to inadequate paralysis. Two of the animals were also stimulated with a flashing light while clicks were presented. The data from the air puff condition were compared with the pre-control by a t test.

8.3.4 Results

In each cat, there was a marked reduction in MG evoked potential amplitude when the clicks were recorded during the presentation of the air puff. Figure 8-2 shows two occasions in which the effect was observed in one cat and Figure 8-3 shows similar effects in another. There is a significant difference between the control and the air puff amplitudes ($t = 3.12$, $p < .05$, $df = 4$).

Figure 8-4 shows a reduction in MG amplitude without any reduction in CN amplitude. Figure 8-5 shows no reduction in an IC response. These results indicate that the effect is occurring at the thalamus. Figure 8-6 shows that no decrement is produced by a flashing light, but a large decrement is produced by an air puff.

8.3.5 Discussion

These data indicate that there is a marked suppression of evoked potentials recorded at the MG when a painful stimulus is given concurrently. Similar changes are not observed when a visual stimulus is used, such as a

Figure 8-2

Averaged evoked potentials recorded at the MG of one cat under Flaxedil. Columns 1 and 2 show three averaged responses: pre-control, air puff and post-control. There is a marked reduction in response amplitude during presentation of the air puff. Amplitude calibration is 50 microvolts.

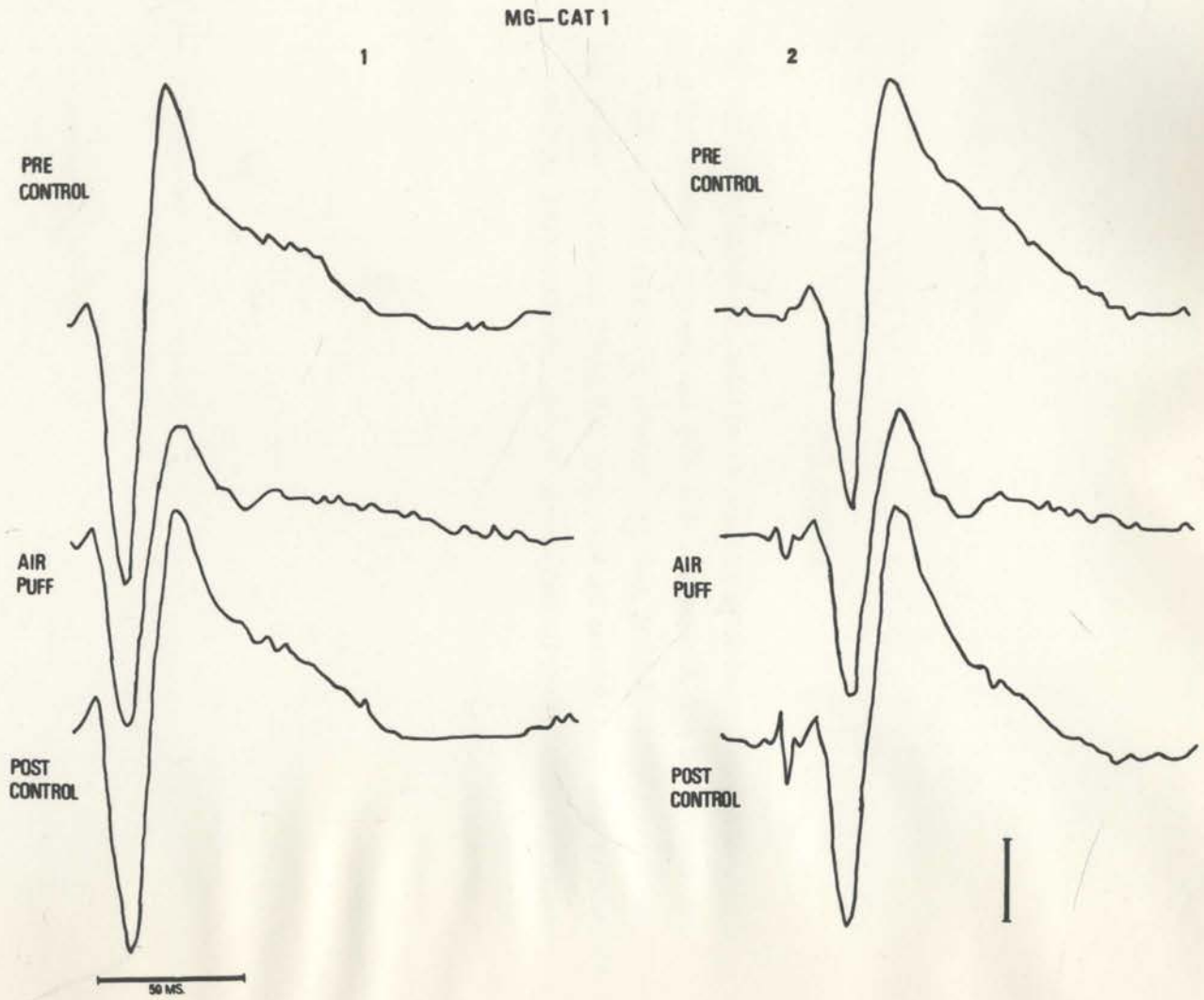

MG—CAT 1
1
2
PRE
CONTROL
AIR
PUFF
POST
CONTROL
PRE
CONTROL
AIR
PUFF
POST
CONTROL
50 MS.

Figure 8-3

Averaged evoked responses to clicks recorded from the MG of one cat under Flaxedil. The top is the pre-control, the middle is taken during the presentation of air puff to the cornea and the bottom is the post air puff control. There is a marked reduction in amplitude during presentation of the air puff.

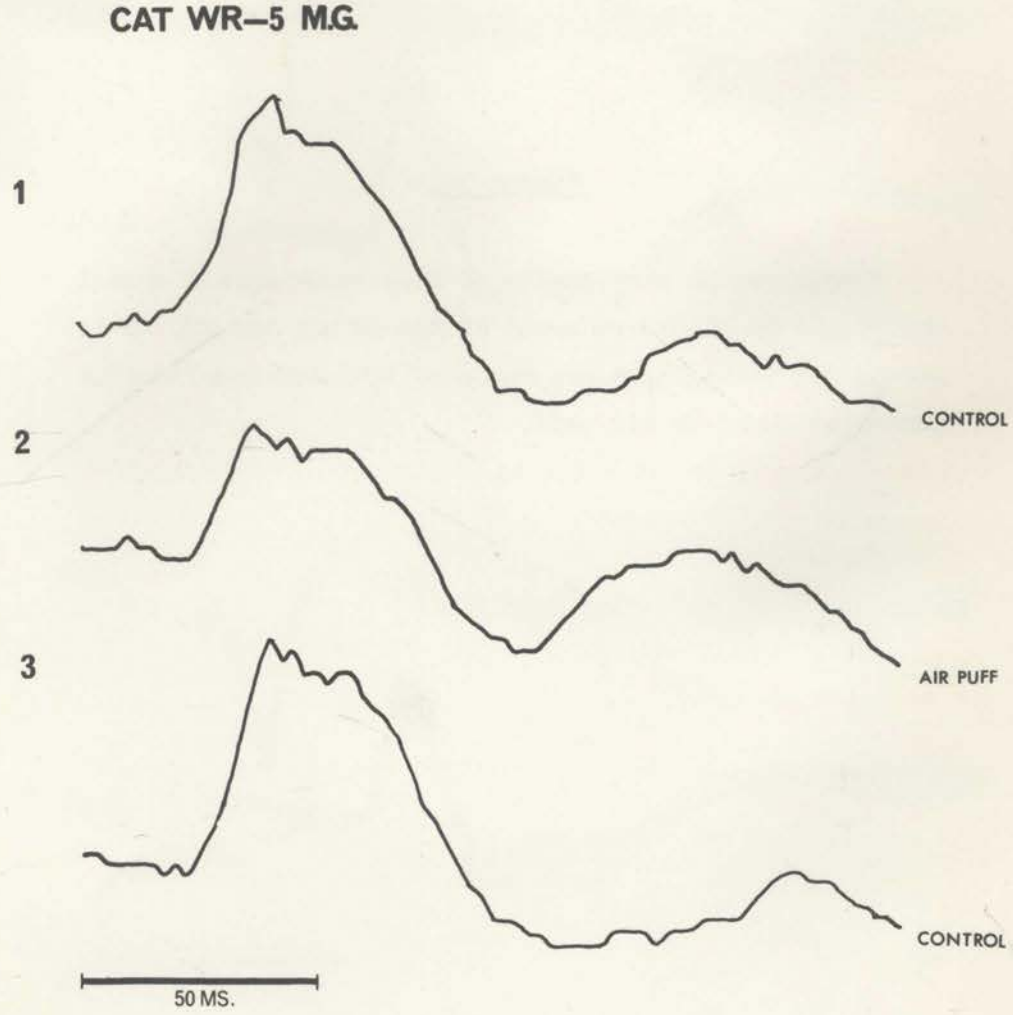

CAT WR–5 M.G.
1
2
3
CONTROL
AIR PUFF
CONTROL
50 MS.

Figure 8-4

Oscilloscope photographs of five superimposed evoked potentials to clicks recorded at the CN and the MG. The bottom two photographs are recorded with the simultaneous presentation of an air puff.

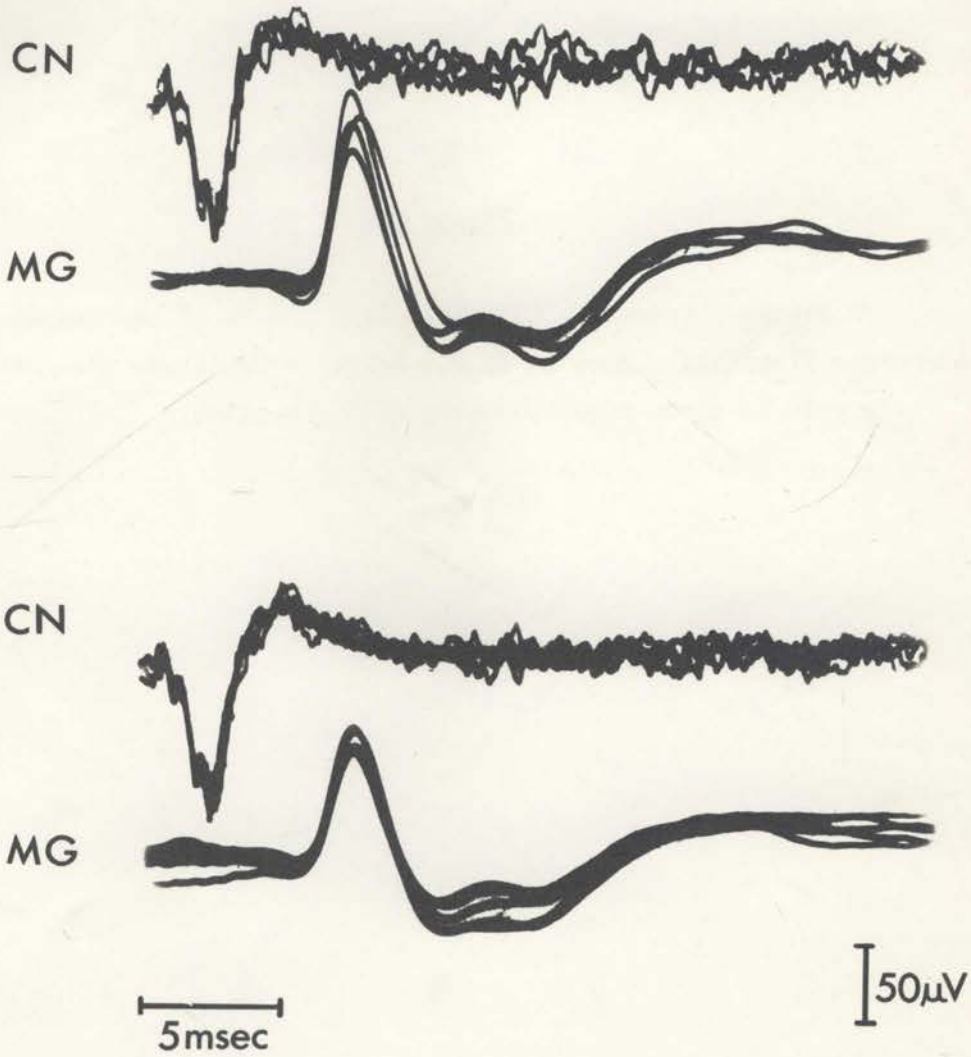

CN
MG
CN
MG
5msec
50μV

Figure 8-5

Averaged responses to clicks from the IC of one animal under Flaxedil. There is no reduction in amplitude when an air puff is given simultaneously with the click.

CAT WR–5 I.C.

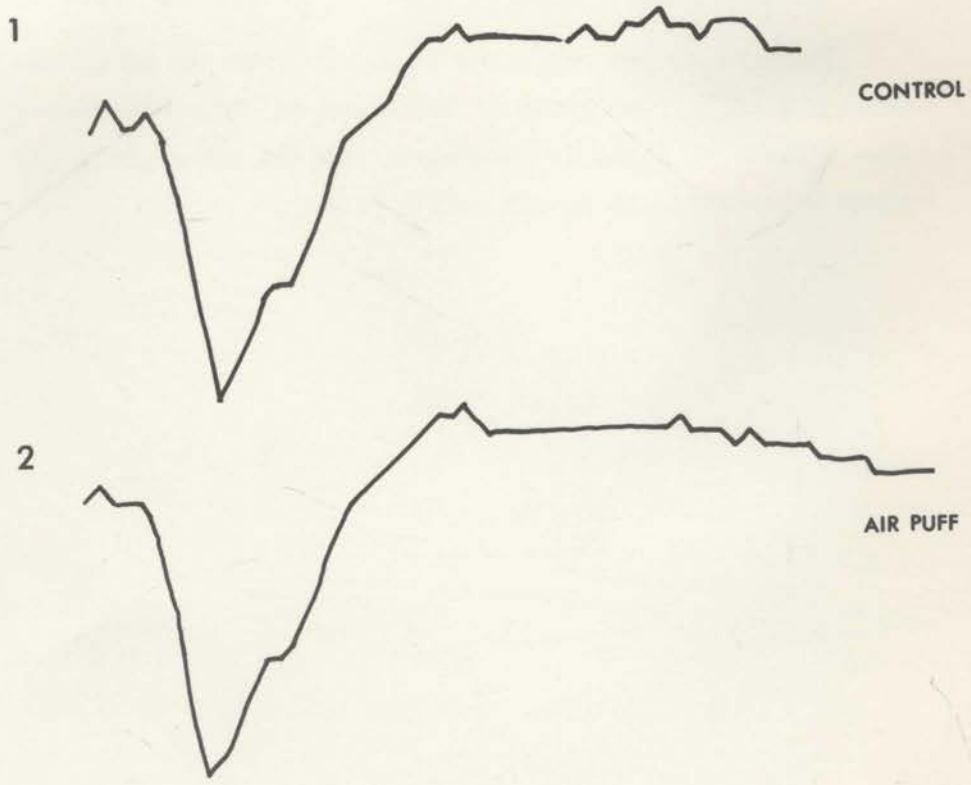
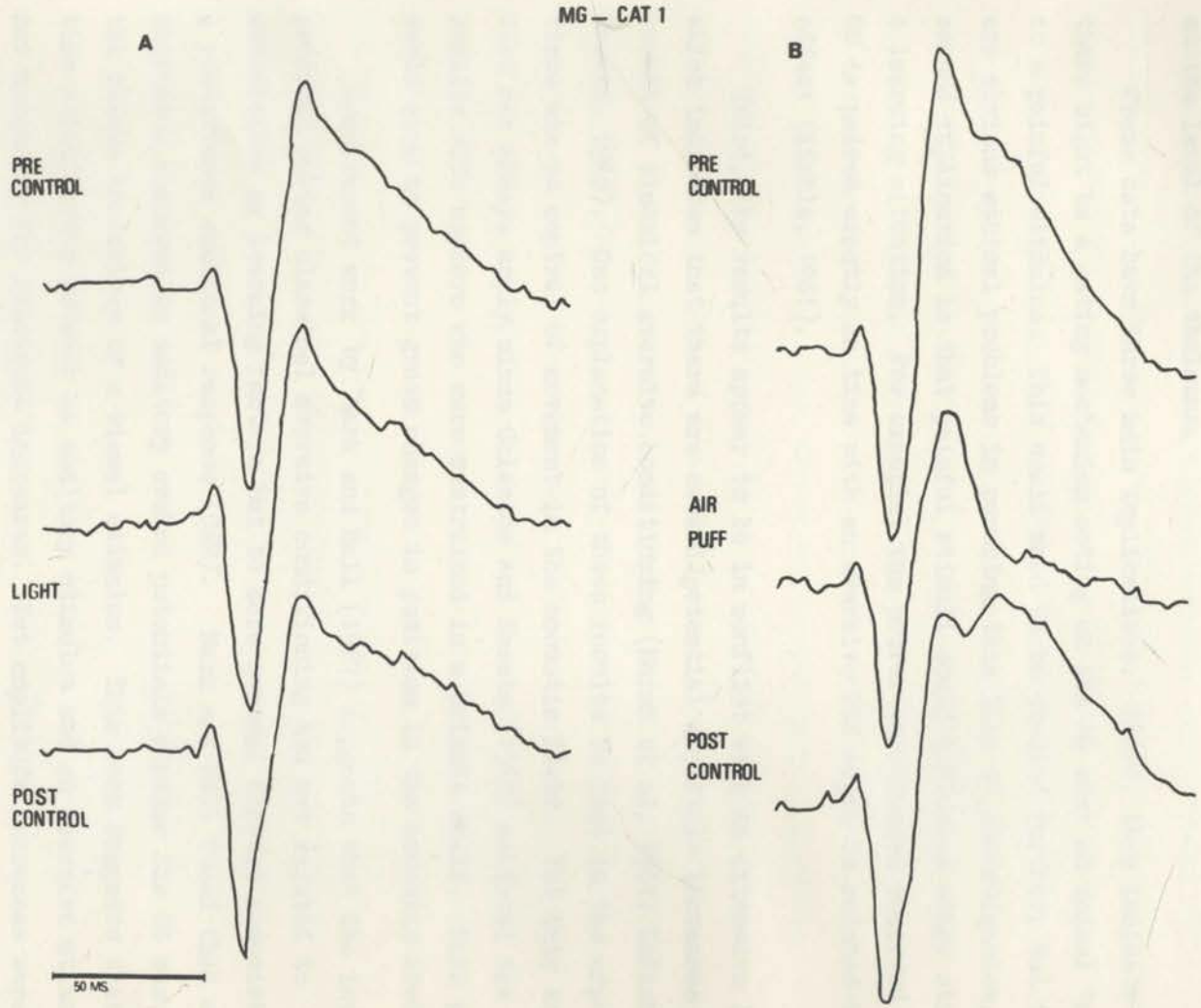


Figure 8-6

Averaged evoked responses to clicks from the MG of one cat under Flaxedil. One group of responses (A) shows no reduction when a flashing light is presented, but the other group (B) shows a reduction to an air puff.


MG — CAT 1
A
PRE
CONTROL
LIGHT
POST
CONTROL
50 MS
B
PRE
CONTROL
AIR
PUFF
POST
CONTROL

distracting stimulus. These results are in agreement with the results of Experiment 16, in which no attention decrements were observed to visual stimuli providing the animal was motionless. The suppression does not occur at the CN and the IC, and this indicates that it is due to a mechanism acting at the level of the thalamus.

These data have three main implications. First, they indicate that there might be a gating mechanism acting on the MG when an animal "attends" to a painful stimulus. This would need to be studied further, but there are obvious ethical problems in pursuing this line of investigation. The second implication is that painful stimuli could influence other stimuli in a learning situation. For example, the poorer performance observed when a CS is paired exactly in time with an aversive UCS might be related to this effect (Kimble, 1961).

Third, the results appear to be in conflict with an extensive literature which indicates that there are evoked potential amplitude increases as a result of classical aversive conditioning (Marsh et al, 1961; Galambos and Sheatz, 1962). One explanation of these results is that in the experiments there was no control of movement in the acoustic field. Yet this argument does not always apply, since Galambos and Sheatz (1962) achieved the same results with monkeys who were restrained in a primate chair. This procedure would seem to prevent gross changes in position in the acoustic field.

Some recent work by Mark and Hall (1967) suggests that the increases produced during classical aversive conditioning are not related to associative or learning factors but to more general factors associated with a conditioned emotional response (CER). Mark and Hall found that amplitude increases occurred in auditory evoked potentials whether the CS was either the clicks themselves or a visual stimulus. This work suggests that a strict time relationship between an auditory stimulus and an aversive stimulus is not necessary for amplitude increases. Yet amplitude increases were not observed in either Experiment 15 in which the shock followed the auditory stimulus by 450 msecs, or in the present experiment in which the aversive stimulus was applied continually.

However, the results of the present experiment need not be in conflict with other work, since in all these studies the evoked potential was not

measured during the presentation of the aversive stimulus. In fact, it would be difficult to obtain a meaningful measure at such a time unless the animal were paralysed. This is because the action of the aversive stimulus would activate the middle ear muscles and in this way produce amplitude decrements. However, it might be expected that an increase in amplitude would occur after the aversive stimulus was removed. This did not occur in either Experiment 15 or the present experiment.

One explanation of these results is that the effects are associated with the paralysing action of Flaxedil. It is possible that a necessary condition for the amplitude increases is that the aversive stimulus should induce a conflict in the animal. For example, in the CER situation a conflict is set up between making a response (usually a bar press) and responses induced by the aversive stimulus. When the animal is paralysed, of course, this conflict cannot develop.

Galin (1965) reported that non-contingent pairing of shock with white noise led to depression of integrated activity recorded at the IC. There was a marked reduction in this activity during noise presentation, but the interesting point is that the depression lasted for days after the shocks were terminated. In contrast to Galin's findings, a depression was not observed at the IC in the present experiment during the presentation of the aversive stimulus. The difference between the two sets of results need not be important, since the recording methods were so different. It is possible that a depression occurs in integrated activity which is not present in an evoked potential produced by a click.

CHAPTER 9

9.0 Conclusion

This dissertation set out to examine the relationship between the amplitude of auditory evoked potential responses to stimuli presented in two situations. The first involved a non-attending or habituation situation in which the organism is stimulated in a repetitive manner by auditory stimuli. The second has been described as an attending (directing of receptors) situation. Here, an organism is presented with auditory stimuli while it is directing its receptors to stimuli of other modalities.

The conclusion for the first situation is that changes to repetitive stimuli in auditory evoked potential amplitudes at the CN, IC and MG do not represent changes due to an habituation process. They appear to represent changes due to a refractory process based on inhibition. It would seem on the basis of these results and the work of Wall (1967) that attempts to determine whether there is an habituation mechanism in the auditory pathway should be based on single-unit recording or perhaps multi-unit recording. Wall's (1967) work suggests that habituation might be based on a small number of cells. In that case, even multi-unit recording might be too coarse. However, it might be possible that there is a structural relationship which would show up with multi-unit recordings. For example, Wall (1967) showed that his novelty cells were restricted to a lamina in the area in which he was recording. If this were the case in the auditory system, then multi-unit recordings could be valuable. They have a number of advantages over chronic single-unit recordings, in that implantation is easier and the electrode does not need to be moved in search of cells.

(Buchwald et al (1966) have found indications of habituation using multi-unit recordings. However, they have used 1500 cps tone burst of 1.5 sec duration which could lead to a confounding of adaptation and habituation effects. It is hard to separate out these factors in this experiment, as no information is provided about inter-stimulus interval.)

The conclusion for the second situation is that auditory amplitude changes are not related to "attention" to a visual stimulus. Such changes

only occur when the animal is moving, which indicates that the decrements are due to activation of the middle ear muscles. Similar results have been obtained by Starr (1964). Furthermore, it appears that an aversive stimulus can lead to amplitude reductions in evoked potentials recorded in the thalamus. This observation could indicate an "attention" effect due to painful stimuli.

These results, taken in conjunction with other studies (Galin, 1965; Mark and Hall, 1967), indicate that under a variety of conditions auditory evoked potentials in one sensory pathway can be modified by the effects of stimuli in other pathways. This can happen indirectly (CER) or directly (painful stimuli). The next step in the study of attentional mechanisms could be the study of the mechanism behind both the increases and decreases in amplitude. The technique developed by Burke and Sefton (1966) could be the most suitable instrument for such a study. Using electrical stimulation of the optic pathway, they were able to separate out the pre- and postsynaptic components of lateral geniculate evoked potentials. This technique could show how and where the above amplitude changes are taking place.

ADDENDUM

Since this dissertation was typed, several papers have been published which bear on the topic. Cook, Ellinwood and Wilson (1968) have reported decrements to repetitive stimulation in evoked potentials recorded in the auditory cortex. They found that the size of the decrement was a function of the rate of stimulation and that similar decrements were obtained with regular and random patterns of stimulation. This work confirms some of the data reported in this dissertation.

W.D. Wickelgren (1968a, 1968b, 1968c) has reported decrements to repetitive stimulation at the MG and auditory cortex, but no change in potentials recorded at the CN and IC. However, Wickelgren has employed a most unusual method of repetitive stimulation. He presented a train of 32 clicks at 5/sec, and each train was repeated every minute. This procedure is equivalent to bursts of stimulation interspersed with periods of spontaneous recovery. On the basis of the recovery data reported in this dissertation, it might be predicted that very little decrement would be present at the CN and IC if 55 seconds were allowed to elapse between bursts of stimuli. Wickelgren did not use a control level that was obtained independently of the habituation trains of clicks. Since the decrements have a rapid onset, it is possible that the magnitude of the decrement was obscured by decrements occurring within the trains of clicks. Experiment 12 indicates that decrements can be seen in the second evoked potential to a train of clicks when the stimulation rate reaches five per second.

Ritter, Vaughan and Costa (1968) reported habituation to auditory stimuli in averaged evoked responses recorded from scalp electrodes in human subjects. They found that tones delivered every two seconds produced a rapid decrement in amplitude. They did not find these changes with stimuli delivered at the rate of 1/10 sec. They also failed to find dishabituation of the decrement. They concluded that the rapid drop only appeared to be habituation and that it really reflected refractoriness within the auditory system. Although one must be cautious in relating scalp electrode recordings to subcortical evoked potentials, the correspondence between these data and the experiments reported in this dissertation is striking.

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