1971

Comparative Studies Of The Relation Of Hippocampal Electrical Activity To Behavior

Ian Quentin Whishaw

COMPARATIVE STUDIES OF THE RELATION OF HIPPOCAMPAL ELECTRICAL ACTIVITY TO BEHAVIOR

by

Ian Quentin Addison Whishaw

Department of Psychology

Submitted in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

Faculty of Graduate Studies

The University of Western Ontario

London, Canada

February, 1971

ABSTRACT

The purpose of the research was to investigate the nature of hippocampal electroencephalographic activity (EEG) in rodents (rat and Mongolian gerbil) and cats. EEG was recorded during naturally occurring behaviors (walking, feeding, grooming, etc.), formal locomotor tests (e.g., wheel running) and conventional learning tests (bar pressing and shock avoidance).

Three types of electrical patterns were recorded: (1) rhythmical slow activity (RSA), (2) large amplitude irregular activity (LIA), and (3) small amplitude irregular activity (SIA). RSA accompanied voluntary movement (walking, jumping, swimming). LIA was associated with automatic activities (chewing food, lapping water, face washing, etc.) and alert immobility. SIA was associated with sudden arrest of voluntary movement.

During large movements (run, jump) RSA amplitude was up to six times as great as during small movements (head turn, bar press). During steady movement RSA frequency was constant between 7.5-8.3 Hz regardless of response speed (running), response duration (up to 8 hrs) or movement pattern (walk, trot, gallop, swim). RSA frequency increased with movement initiation and more abruptly initiated movements were associated with higher frequencies (11-12 Hz, 22 in jump) than more gradually initiated movements (8-10 Hz, 11 in jump). During immobility in

a fear conditioning task significantly lower RSA frequencies occurred than during any overt movement (less than 6 Hz). RSA frequency may reflect initiation of movement and maintained movement, while amplitude reflects movement size, suggesting that parameters reflect different mechanisms. This hypothesis was supported by the finding that frequency was normal after a hippocampal fit but amplitude was depressed; frequency varied directly with core temperature (26 to 41°C) but amplitude was constant.

Increases in RSA frequency and amplitude during paradoxical sleep were associated with muscular twitches.

Neither amplitude nor frequency of RSA changed with learning independent of movement.

RSA did not appear to be related to rhythmical activities such as vibrissae movements, heart beat, sniffing, lapping water, chewing, etc, independently of the occurrence of voluntary movements.

The results suggest that the hippocampal EEG reflects the activity of two mechanisms associated with the control of movement. One system generates RSA and initiates voluntary movements, the other suppresses RSA (SIA) and arrests voluntary movement. It was concluded that the hippocampal EEG indicates hippocampal participation in the control of voluntary movement in both sleeping and waking states.

ACKNOWLEDGEMENT

I would like to thank Case Vanderwolf for his critical discussion of the research and the presentation of the results. I would also like to thank Susan Whishaw for her assistance in many phases of the research.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The principal concern in the present thesis is the behavioral significance of the rhythmical slow activity which can be recorded from the hippocampus. In the introduction pertinent hippocampal anatomy will first be outlined. Then some classical theories of behavioral functions of the hippocampus will be briefly described. Finally, the electrophysiology of the hippocampus and the behavioral interpretations of this electrophysiology will be described.

The gross anatomy of the hippocampus has been reviewed extensively several times (Akert and Hummel, 1968; Cajal, 1955; Crosby, Humphrey and Lauer, 1962; Green, 1960, 1964; Lorente de Nó, 1933, 1934; Pribram and Kruger, 1954). A brief outline of the anatomy pertinent to the present investigation may, however, prove helpful.

Structures included in the term hippocampus, as it is used in its most general sense, are the subiculum, presubiculum, parasubiculum, dentate gyrus, Ammon's horn (or the hippocampus proper or hippocampus major) and entorhinal cortex as well as associated pathways. Some authors (Green, 1960) also include the cingulate gyrus, amygdala and septum lucidum. Generally, however, anatomists include only the dentate gyrus, Ammon's horn and the subicular areas and associated pathways but exclude the entorhinal cortex (Crosby, Humphrey and Lauer, 1962).

The hippocampus in sub-primates consists of a dorsal portion, called the dorsal hippocampus, which lies immediately below the lateral

ventricle. It is continuous with a ventral portion, called the ventral hippocampus, lying within the inferior horn of the lateral ventricle. The hippocampus is rolled upon itself with the dentate gyrus lying within the curvature of the outer hippocampus major. Although the hippocampus major and dentate gyrus arise from the same primitive hippocampal formation, they are anatomically different and should be referred to as different structures (Crosby, Humphrey and Lauer, 1962).

The dentate gyrus (or fascia dentata) consists of three cell layers, (1) a layer of plexiform cells, (2) a layer of granular cells and, (3) a layer of polymorph cells. The dendrites of the granular cells go out mainly to the molecular layer, while the axons (or mossy fibers) turn into the polymorph layer to enter the hippocampus major where they synapse on the apical dendrites of the pyramidal cells.

The hippocampus major lies over the dentate gyrus and together they form two interlocking C-shaped segments, so that one end of the curvature of the hippocampus major lies within the curvature of the dentate gyrus. The hippocampus major also contains three layers of cells, (1) a layer of molecular cells, (2) a layer of double pyramidal cells and (3) a layer of polymorph cells. The hippocampus major has further been subdivided into four major fields, primarily on the basis of connections and structure of the pyramidal cells. The fields, CA 1 to CA 4, lie along the curvature of the C of the hippocampus major, with CA 1 located most medially beneath the ventricle and CA 4 located within the curvature of the dentate gyrus (Lorente de Nó, 1934). The main efferent fibers of the hippocampus major are the axons of the pyramidal cells. These axons project toward the ventricle and enter

the alveus (or the main fiber pathway beneath the ependyma of the lateral ventricle). The axons then enter either the hippocampal commissure to synapse with the basal dendrites in the homo-topic region of the contralateral hippocampus, or they enter the fornix. The axons that enter the fornix project to the septum, preoptic area, or the mammillary bodies. Some pyramidal cells, particularly in field CA 3, give rise to collateral axons (Schaeffer collaterals) which project to field CA 1 and terminate in the area of the large apical dendrites of the pyramidal cells.

The major pathway entering the hippocampus is the temporo-ammonic tract. The afferents of the temporo-ammonic tract arise mainly in the entorhinal cortex and divide either to end among the apical dendrites of the pyramidal cells of field CA 1, or enter the dentate gyrus. Fibers from the cingulate gyrus (as part of Papez circuit) may also project to the hippocampus in a similar manner (Green, 1960). Although anatomists have not described fibers projecting from the hippocampus to the entorhinal cortex experimental electrical stimulation of the hippocampus can produce evoked potentials in the entorhinal cortex suggesting such a projection does exist (Adey, Merrillees and Sutherland, 1956). A second pathway, the septo-hippocampal pathway, has received considerable attention since it appears to play a role as a relay between the reticular system and hippocampus by which hippocampal rhythmical activity is activated. The septal-hippocampal afferents arise in the medial septal area, pass through the dorsal fornix to enter either the dentate gyrus and relay onto the granular cells, or to project directly to the pyramidal cells of fields CA 2

to CA 4 (Raisman, 1966).

Recent anatomical evidence (Blackstad, Brink, Haem and June, 1970) has shown that the mossy fibers of the dentate gyrus do not extend into the fornix, but rather have a precise level to level localization on the apical dendrites of the pyramidal cells in fields CA 2 to CA 4. It appears evident, therefore, that the main output from the hippocampus is through the axons of the pyramidal cells from the hippocampus major. In summary, input to the hippocampus major from extra-hippocampal areas is both direct and indirect. Field CA 1 receives afferents directly from the temporo-ammonic tract and is influenced indirectly by the other pyramidal fields through the Schaeffer collaterals. Fields CA 2 to CA 4 receive afferents directly from the medial septal area but can be influenced indirectly by mossy fiber projections from the dentate gyrus granular cells which receive direct input from the entorhinal area via the temporo-ammonic tract. The significance of this highly organized system of connections between the hippocampus major, dentate gyrus and extra-hippocampal structures is uncertain. However, the hippocampus is ideally located and structured to integrate activity from a variety of sources.

Classical Theories of Hippocampal Function

Four classical theories of the role of the hippocampus in psychological functions have arisen historically. Early anatomical investigations of the hippocampus revealed strong connections with the sensory system for smell and, for this reason, the hippocampus was classified as part of the rhinencephalon or olfactory brain.

Subsequent behavioral investigations (Allen, 1940; Swann, 1934) indicated that the hippocampus played little or no part in olfactory discrimination in mammals. Therefore, although the conventional anatomical terminology has been retained, the notion that the mammalian hippocampus is involved in olfaction has been largely abandoned.

Papez (1937) proposed that the hippocampus was involved in emotion, as part of a "circuit" comprising the fimbria, fornix, mammillary bodies, anterior thalamus, cingulate gyrus and entorhinal cortex. Papez' circuit, as this system has come to be called, supposedly elaborated emotional expression and any "break" in the circuit produced emotional disorders. This idea supplied a function for olfactory areas which apparently had no olfactory functions and provided a system for emotional expression for which no neural mechanism had been identified. Papez' (1937) proposal received quite wide acceptance when temporal-lobe damage was shown to produce emotional peculiarities in monkeys, including tameness, hyper-sexuality and compulsive oral tendencies (Klüver and Bucy, 1939). Later, MacLean (1949) suggested that Pepez Circuit provided a neural basis, with the hippocampus as a control center, through which emotional disorders were manifest as psychosomatic illness. Although, Papez' (1937) general proposal proved untenable (Pribram and Kruger, 1954), it led to investigations which demonstrated that certain rhinencephalic areas were involved in emotion (e.g., septum and amygdala). However, no direct emotional functions for the hippocampus have been demonstrated.

A third idea was that the hippocampus was involved in recent memory (Milner, 1966) since bilateral hippocampal resection in man

produced a dramatic amnesic syndrome (Milner and Penfield, 1955). Milner's proposal is generally accepted currently but the amnesic effect of hippocampal removal seems restricted to man. Hippocampal ablation in animals (mainly in sub-primate species) has resulted in behavioral changes which suggest that the hippocampus has a function of inhibiting response tendencies (for reviews see, Douglas, 1967; Kimble, 1968). Hippocampectomized animals are able to learn simple discriminations and retain them for long periods of time, but the responses do not extinguish readily and large deficits occur on reversal training. Animals also show deficits in passive avoidance tasks and on maze learning problems. This evidence suggests that in a normal animal the hippocampus operates to suppress behavioral responses which are no longer reinforced, thus, permitting elaboration of new responses. The way in which the hippocampus selectively inhibits response tendencies is not known, although, a number of hypotheses have been advanced (Douglas, 1967).

The Origin of Hippocampal Electrical Activity

The electrophysiology of the hippocampus has been reviewed a number of times (Green, 1960, 1964; Stumpf, 1965a; Vanderwolf, 1969b), and it appears that a variety of structures participate in the generation of various hippocampal waveforms. The following sections will describe: (1) the types of electrical activity which can be recorded from the hippocampus, (2) species differences, (3) the source of electrical activity, (4) the cellular basis of electrical activity, (5) extra-hippocampal structures responsible for activation of

produced a dramatic amnesic syndrome (Milner and Penfield, 1955). Milner's proposal is generally accepted currently but the amnesic effect of hippocampal removal seems restricted to man. Hippocampal ablation in animals (mainly in sub-primate species) has resulted in behavioral changes which suggest that the hippocampus has a function of inhibiting response tendencies (for reviews see, Douglas, 1967; Kimble, 1968). Hippocampectomized animals are able to learn simple discriminations and retain them for long periods of time, but the responses do not extinguish readily and large deficits occur on reversal training. Animals also show deficits in passive avoidance tasks and on maze learning problems. This evidence suggests that in a normal animal the hippocampus operates to suppress behavioral responses which are no longer reinforced, thus, permitting elaboration of new responses. The way in which the hippocampus selectively inhibits response tendencies is not known, although, a number of hypotheses have been advanced (Douglas, 1967).

The Origin of Hippocampal Electrical Activity

The electrophysiology of the hippocampus has been reviewed a number of times (Green, 1960, 1964; Stumpf, 1965a; Vanderwolf, 1969b), and it appears that a variety of structures participate in the generation of various hippocampal waveforms. The following sections will describe: (1) the types of electrical activity which can be recorded from the hippocampus, (2) species differences, (3) the source of electrical activity, (4) the cellular basis of electrical activity, (5) extra-hippocampal structures responsible for activation of

electrical activity in the hippocampus and (6) the role of the septum as a pacemaker for certain wave forms. Since the primary concern of the present thesis is the slow activity of the hippocampus, the following description will deal mainly with the rhythmical slow activity (RSA).

Types of Hippocampal Electrical Activity. A number of types of electroencephalographic (EEG) activity can be recorded from the hippocampus. Stumpf (1965b) has described at least three slow rhythms and a fast rhythm in the hippocampus of the rabbit. Slow activity included (1) a large amplitude sinusoidal rhythmical slow activity (RSA), (2) a large amplitude irregular slow activity (LIA) and (3) a depressed activity or small amplitude irregular activity (SIA). The names and abbreviations of slow wave forms are those used by Vanderwolf (1969a, 1969b). The classical terminology for RSA has been the theta rhythm, since the frequency of this activity was believed to be between 4-7 Hz (Green and Arduini, 1954). However, since it appears that the frequency may vary between 2-12 Hz in different species, the term RSA is preferable. Faster waveforms can also be recorded from the hippocampus but these appear to be independent of slower patterns (Eidelberg, White and Brazier, 1959), may be activated by different mechanisms (Stumpf, 1959b; Torii, 1961; Yokota and Fujimori, 1964) and may also be generated by different cell populations (Green, Maxwell, Schindler and Stumpf, 1960). Most behavioral and EEG investigations have been directed at the significance of RSA.

Species Differences. RSA has been studied mainly in sub-primate species: rabbits, rats, guinea pigs and cats and dogs. It has been observed that RSA is readily obtained from the phylogenetically lower species, more difficult to obtain from cats, and is rarely seen or absent altogether in monkeys (Gergen, 1967; Green and Arduini, 1954). Finally, there are no reports of RSA having been recorded from the hippocampus of man. The increased difficulty of recording RSA from the "higher" species has led to the speculation that there is a phylogenetic change in hippocampal EEG (Parmeggiani, 1967). Paralleling this EEG change is the increased development of the neocortex as well as an apparent functional change revealed by the differential effects of hippocampal ablations in man and sub-primates. On the basis of these lines of evidence it is tempting to speculate that the function served by the hippocampus in lower species is taken over by the developing neocortex. However, it should be noted that there is surprisingly little change in the anatomy of the hippocampus from mouse to man (Lorente de Nó, 1933). Further, the electrical activity in primates may not have been studied under optimal conditions; for example, chances of recording RSA are maximized when movements occur (Vanderwolf, 1969a). Thus, it is difficult to conclude on the basis of present evidence that there really are more than superficial differences in EEG activity and it may be hazardous to conclude that assumed electrical differences underlie a functional change.

The Source of RSA. The occurrence of RSA does not seem to be limited to the hippocampus. In different studies RSA has been recorded from a number of cortical and subcortical structures including the

neocortex, thalamus, hypothalamus and brain stem (Brown, 1968; Elazar and Adey, 1967; Grastyán, Lissák, Madarász and Donhoffer, 1959; Green and Arduini, 1954; Holmes and Adey, 1960; Parmeggiani and Zanocco, 1963; Vanderwolf and Heron, 1964). Evidence, however, suggests the source of RSA is the hippocampus. The largest amplitude RSA occurs in this structure (Green and Arduini, 1954; Vanderwolf, 1969a; Petsche and Stumpf, 1960), and lesions to the septum which abolish RSA from the hippocampus also abolish RSA from other structures (Parmeggiani and Zanocco, 1963). Further, it has been found that RSA occurs first in the hippocampus and then passes caudally and ventrally to other structures (Petsche and Stumpf, 1960). The significance of RSA in sites other than the hippocampus is uncertain, but it may indicate that these structures participate at times in a common function.

Within the hippocampus the generator of RSA has been found to lie within the pyramidal cell layer of the hippocampus major (Green, Maxwell, Schindler and Stumpf, 1960). When a gross recording electrode was fixed below the pyramidal cell layer in one hippocampus, and a microelectrode lowered vertically into the hippocampus nearby, or into the contralateral hippocampus at a homotopical point, activities of the RSA type, recorded from the two electrodes were at first 180° out of phase with one another. As the microelectrode was lowered, the amplitude of RSA recorded from it increased gradually, then decreased, and disappeared at a point just below the pyramidal cell layer. Deeper penetration of the microelectrode resulted in a reappearance of RSA which was now in phase with RSA recorded from the fixed electrode, and increased steeply in amplitude as the microelectrode moved downward.

It was, therefore, concluded that the generator of RSA lay between the distal ends of the apical and basal dendrites.

Neuronal Mechanisms of RSA. The cellular changes underlying the generation of RSA are not well understood. It has been found that some pyramidal cells fire in phase with the rhythm, but others do not (Green, 1964). However, the rhythm is presumably not directly related to action potentials since it is of maximum amplitude when recorded in the layer of apical dendrites of the pyramidal cells, where action potentials are rarely recorded.

A number of explanations have been proposed to account for RSA. Euler and Green (1960) recorded intracellularly the activity of single pyramidal cells and found that during depolarization a series of spikes appeared which declined in amplitude and then ceased altogether. This was followed by hyperpolarization. The time course of these processes suggested that alternating periods of depolarization and hyperpolarization in a large number of cells could account for RSA.

A somewhat similar explanation has been proposed by Spencer and Kandel (1962; see also Kandel, Spencer and Brinley, 1961). These authors recorded intracellularly in the hippocampal pyramids and found that when cells fired in bursts, the membrane potential did not return to the baseline and new spikes originated from partially depolarized cells; this led to very rapid firing, but limited the duration of a burst to less than 20 msec. Subsequently, long (200-300 msec) inhibitory postsynaptic potentials appeared and produced a further inhibition of the cell. These were thought to be mediated by recurrent collaterals from neighbouring cells which might therefore, play a key role in generating

rhythmical activity. The recovery period of spike parameters following a spike or burst of spikes was similar in duration to the period of an RSA wave and in addition the recurrent inhibitory effects might provide a mechanism for the possible recruitment of neighbouring cells into the rhythm. According to Eccles (1964) the recurrent inhibition is due to basket cells which synapse on the pyramidal cell soma (Andersen, Eccles and Lyning, 1963) acting in much the same way as the Renshaw cells in the spinal cord.

Fujita and Sato (1964), however, have proposed that RSA may originate from summed excitatory post-synaptic potentials. The evidence for this is that an "intracellular theta wave" can be recorded as a rhythmical fluctuation of the membrane potential (up to 10 mV) which is in phase with the extracellular rhythm. Hyperpolarizing the cell causes inhibitory post-synaptic potentials to become depolarizing and should lead to a phase reversal of the intracellular rhythm if this rhythm is due to an inhibitory input. However, this does not occur. Thus, the precise mechanism of rhythmical waves in the hippocampus remains somewhat obscure.

Activation of RSA. Although the neurophysiological basis of RSA is imperfectly understood, the evidence consistently supports the idea that RSA is generated by the pyramidal cells. In addition, all evidence is in agreement that some extra-hippocampal input is necessary to activate the rhythm and that activation of RSA is due to ascending excitation from the brain stem reticular formation and other subcortical structures. By the combined use of the techniques of electrical

stimulation (which activates RSA) and lesions (which abolish RSA), a pathway has been traced through the posterior medial hypothalamus, medial hypothalamus, medial preoptic area and medial septum and through the dorsal fornix to the hippocampus, by which RSA is activated (Corazza and Parmeggiani, 1963; Green and Arduini, 1954; Petsche, Stumpf and Gogolák, 1962; Torii, 1961; Yokota and Fujimori, 1964). An alternate activating pathway has also been found to run through the medial thalamus to the medial septum (Eidelberg, White and Brazier, 1959; Green and Arduini, 1954; Kawamura, Nakamura and Tokizane, 1961).

Activation of RSA is not the only hippocampal response which can be obtained in response to subcortical stimulation. Electrical stimulation can also produce a suppressed pattern of electrical activity. This suppressed activity appears to be due to the inhibition of slow waves in the hippocampus. This inhibition indicates that a second system in the brain is capable of antagonizing the RSA producing system. Stimulation of the lateral hypothalamus and lateral preoptic area produce suppression, suggesting that the pathway for this system lies just lateral to the main RSA activating pathway (Stumpf, 1965a; Torii, 1961; Yokota and Fujimori, 1964). Although it is clear that the RSA activating system must act on the hippocampus, the suppressing system may act either on the hippocampus or on the activating pathway, possibly at the level of the medial septum (Stumpf, 1965a, b).

The Role of the Septum in Pacing RSA. A number of lines of evidence indicate that the septum plays an important part in the activation of RSA. Bilateral lesions in the medial septum abolish RSA from the hippocampus and unilateral lesions abolish RSA from the

ipsilateral hippocampus (Green and Arduini, 1954). Thus, the medial septum acts as a funnel for thalamic and hypothalamic pathways which activate hippocampal RSA. Evidence, however, has been obtained which indicates that the septum may also pace the hippocampal rhythm. Single unit recordings obtained from septal cells show that some cells fire in phase with hippocampal RSA even though RSA cannot be recorded from the septum (Petsche, Stumpf and Gogolák, 1962; Stumpf, Petsche and Gogolák, 1962). Although the phase relation was not the same in all cells, any one cell maintained a constant phase relation to RSA. The activity of these cells did not appear to be secondary to RSA since their rhythmical discharge continued through hippocampal postictal depression as well as after the administration of lysergic acid diethylamide, urethane and nicotine had disrupted RSA. A direct relation has also been found between the activity of these cells and RSA frequency in response to electrical stimulation of the reticular system (Gogolák, Petsche, Sterc and Stumpf, 1967). These findings suggest that the septum acts not only as a relay between the brainstem and hippocampus but also may be responsible for pacing the hippocampal rhythm.

In summary, activation of the brainstem is passed to the septum through either the medial hypothalamus or the medial thalamus. Some cells in the septum pace their activity in response to activation and recruit a rhythmic activity in the hippocampus. This rhythmic response is generated over the dipole of the pyramidal cells of the hippocampus major. However, other mechanisms in the brainstem can suppress RSA by either acting on the pacing cells in the septum or

on the hippocampus directly.

The Functional Significance of RSA

A main controversy concerning the significance of RSA is based on opposing views of whether RSA represents an activation of the hippocampus or an inactivation of the hippocampus.

In the earliest studies in which RSA was observed, it appeared to be a spontaneous electrical rhythm (Gerrard, Marshall and Saul, 1939; Saul and Davis, 1933). Later it was found that the rhythm could be induced by auditory and painful stimuli (Jung and Kornmuller, 1939). The first systematic investigation was made by Green and Arduini (1954) who found that any sensory stimulus could activate the rhythm. They observed that RSA was also closely associated with the behavioral arousal of their animals, as well as "neocortical arousal" or the appearance of low voltage fast activity (desynchronization) in the neocortex. Both desynchronization of the neocortex and synchronization of the hippocampus were produced by sensory and electrical brain stimulation, and both were absent when the animals were resting or after they had been injected with pentobarbital sodium. It was also found that novel stimuli were effective in activating these patterns in the two structures but habituation was obtained with repeated stimulation. Any change in a stimulus again made it effective. Since it was recognized that desynchronization of the neocortex signified arousal or activation of that structure (Moruzzi and Magoun, 1949), the concomitant appearance of RSA in the hippocampus suggested that it was in fact a pattern of activation. Green and Arduini (1954), therefore, concluded that RSA was a sign of hippocampal arousal.

The interpretation that RSA is a hippocampal sign of arousal has not been supported by Grastyán, Lissák, Madarász and Donhoffer (1959). On the contrary, they suggested that RSA was a sign of an inactivated rather than an activated hippocampus. In support of this notion they demonstrated that RSA was associated with orienting (looking around) in cats during the initial stages of conditioning on either an appetitive approach response or a shock avoidance response. When the responses were well learned orienting was suppressed and the hippocampus desynchronized during performance. The correlation between learning and hippocampal desynchronization suggested that the hippocampus functioned to suppress orienting and thus permit performance of the correct response. These results indicated that desynchronization of the hippocampus was a sign of hippocampal activation just as desynchronization of the neocortex indicated that that structure was activated. This idea was further supported by the observation that when the cats were aroused by a novel stimulus when resting a desynchronized pattern appeared in both structures. In addition, the authors argue that the classical notion of slow activity is that it represents a sign of inactivation in a neural structure.

Whether RSA disappears from the hippocampus or not during the performance of learned responses has become a point of contention. Hippocampal RSA has been observed continuously during the performance of an active avoidance response in rats (Vanderwolf and Heron, 1964; Vanderwolf, 1969a), a Sidman-avoidance response in rats (Bremner, 1964) and an appetitive approach response in cats and rats (Adey, Dunlop and Hendrix, 1960; Holmes and Adey, 1960; Elazar and Adey, 1967; Pickenhain

and Klingberg, 1967) and both a bar press response and appetitive approach response in dogs (Lopes da Silva and Kamp, 1969; Yoshii, Shimokochi, Miyamoto and Ito, 1966). Other studies show that RSA can disappear during performance. Holmes and Adey (1960) found that RSA dropped out of hippocampal leads but not entorhinal leads while Adey, Dunlop and Hendrix (1960) found RSA dropped out of ventral but not dorsal hippocampal leads during training. Further, Grastyán, Karmos, Vereczkey and Kellenyi (1966) repeated the original Grastyán, Lissák, Madarász and Donhoffer (1959) study with essentially the same results and others (Bennett, 1970; Rhodes, 1969) have observed a similar disappearance of RSA in cats. The disappearance of RSA does not appear to be limited to cats for Routtenberg (1968) reported the disappearance of RSA in exploring rats following habituation to a novel environment.

Despite the contradictory evidence concerning electrical activity in the hippocampus during learning, the conclusion by Grastyan and his group has received support from studies in which the hippocampus has been ablated. Hippocampectomized animals are unable to inhibit previously learned response tendencies or exploratory tendencies (Kimble, 1969). In addition, depression of the hippocampus following a fit is accompanied by orienting and disrupted performance (MacLean, 1957). Since both lesioned and functionally ablated animals display behaviors which would presumably be suppressed in a normal animal, it does suggest that the role of the hippocampus is inhibitory. The finding that RSA can occur throughout training in some tasks is not seen as an inconsistency (Douglas, 1967). Although Adey, Dunlop and Hendrix (1960) found that RSA did not disappear during the performance of a

two-choice discrimination task, Douglas (1967) has pointed out that this type of task does not require a functional hippocampus. In fact, animals with hippocampal lesions can perform a two-choice discrimination as well as normal control animals (Kimble, 1963; Kimble and Pribram, 1963). Therefore, if this type of logic is accepted, it appears possible to interpret RSA as a sign of hippocampal inactivity despite reports that such activity can occur throughout training.

The Relation of RSA to Information Processing and Attention

Apart from the controversy over whether the presence of RSA signifies an active or inactive hippocampus, other interpretations of the significance of RSA have been made. The first considers that certain phase changes may be related to information processing, while the second suggests that changes in frequency may be related to attention.

Changes in phase relations of RSA have been observed between early and late learning (Adey, Dunlop and Hendrix, 1960). During initial training on a two-choice discrimination task, RSA in areas CA 2 and CA 4 of the dorsal hippocampus lead entorhinal activity by about 20-35 msec. In late training entorhinal activity led by about 65 msec. This indicated that the passage of waves was from the dorsal hippocampus to the entorhinal area in early training, while in later training, passage of waves would proceed from the entorhinal area to the dorsal hippocampus. This change suggested that the hippocampus may play a role in information processing as a phase comparator. This observation is not entirely in accord with other studies of hippocampal

function. Considerable evidence indicates that the septum acts as a pacemaker for RSA (Petsche, Stumpf and Gogolák, 1962; Stumpf, Petsche and Gogolák, 1962) and removal of the entorhinal area does not interfere with hippocampal RSA (Green and Arduini, 1954). It has also been shown that RSA spreads from its initiation in the dorsal hippocampus to other structures (Petsche and Stumpf, 1960). Although none of these studies precludes the spread of RSA from the ventral hippocampus to the dorsal hippocampus, no independent evidence supports such a proposition. It may be argued, of course, that the latter studies did not employ well trained animals, but until supporting evidence shows that RSA can be produced by activation of the temporo-ammonic path, the criticism remains valid.

In other studies (Elazar and Adey, 1967) changes in frequency of RSA have been associated with attentional processes. It was found that 6 Hz RSA is associated with correct responding in a visual discrimination task, while slower frequencies are associated with responses during early training and incorrect responses during later training. The higher frequency presumably indicates an attentional process is active which is necessary for performance of the correct response. Other studies (Bremner, 1968; Bremner, 1970; Bremner and Ford, 1968; Ford, Bremner and Richie, 1970) have also suggested that changes in RSA frequency during conditioning and habituation are consistent with the interpretation that the frequency of RSA may be related to attentional processes.

Indirect evidence from studies in which RSA is disrupted seems to support the idea that RSA is related to attention. Subthalamic

lesions made in well trained cats resulted in postoperatively sluggish locomotion, poor discrimination performance as well as slower less regular RSA. However, locomotion, discrimination, and RSA recovered together (Adey, Walter and Lindsley, 1962). Similarly, injections of n - ethyl - 1 - phenylcyclohexylamine monohydrochloride and 1 - (phenylcyclohexyl) pipevidine monohydrochloride abolished RSA and interrupted T-maze performance but again both parameters recovered together (Adey and Dunlop, 1960). These findings seem to indicate that the experimental manipulations disrupted performance by disrupting RSA. Thus, the deficits may have been due to disruption of attentional mechanisms. The fact that performance recovered when RSA returned to normal seems to indicate that the deficits were not due to amnesia.

Whether an attentional hypothesis receives support from future studies or not, it nevertheless seems clear that RSA or some underlying mechanism is necessary for the correct performance of some learned responses. A similar conclusion has been advanced by Gray and Ball (1970).

Hippocampal Electrical Activity and Movement

In view of past speculations on hippocampal function, it seems surprising that evidence should be found relating hippocampal EEG to movement. Evidence, however, has been obtained which suggests that different patterns of electrical activity are related to certain kinds of movement.

The first evidence relating hippocampal EEG to movement was obtained from acute studies in which the electrical stimulation of

brain points which produced either suppression of RSA or activation of RSA were assessed for their effects on reflexes (Yokota and Fujimori, 1964). Stimulation of the amygdala, preoptic area and ventromedial tegmentum which suppressed RSA also suppressed monosynaptic and polysynaptic reflexes. Stimulation of other sites in the medial hypothalamus, medial preoptic area and dorsolateral tegmentum produced RSA and facilitated spinal reflexes.

In later studies on freely moving rats a close relation between RSA and movement has been observed. On the basis of continued observance of RSA during performance of an active-avoidance response, Vanderwolf and Heron (1964) suggested that RSA was related to movement rather than to learning or attention. Subsequently, (Vanderwolf, 1967, 1969a) this analysis was extended. RSA was found to occur during movements of rearing, walking, and handling of food by rats. RSA also occurred when the animals made postural adjustments between acts of grooming different parts of their body or when they made discrete head movements. The close relation between RSA and movement also appeared to extend to amplitude and frequency of RSA. For example, small movements of the head or movements of the paws when an animal was handling food were associated with lower frequencies of RSA than movements of walking or rearing and RSA was found to rise in frequency when rats initiated a movement of jumping out of a box. Smaller body movements also appeared to be related to smaller amplitudes of RSA than larger body movements. In addition, there was no relation between amplitude and frequency of RSA within movement patterns. A similar relation between RSA and movement has been found in the guinea pig (Sainsbury,

1970).

Large amplitude irregular activity (LIA) as well as RSA has been found to correlate with movement patterns. Movements such as chewing, lapping of water, scratching and face washing in rats (Pickenhain and Klingberg, 1967; Routtenberg, 1968; Vanderwolf, 1967, 1969a), foot stomping in the Mongolian gerbil (Kramis and Routtenberg, 1969), and genital licking, pelvic thrusting, and burring (a type of vocalization) in the guinea pig (Sainsbury, 1970) are accompanied by LIA. However, LIA also occurs during alert immobility (Vanderwolf, 1967, 1969a) and immobility associated with isometric muscle tension such as occurs when a rat hangs by its paws from a ledge (Vanderwolf, 1969a).

To account for the differences in electrical activity associated with different behaviors, Vanderwolf (1969a) has suggested that behaviors can be divided into at least two general categories; those which are automatic and those which are voluntary. Automatic activities include chewing, lapping water, face washing and scratching. Those which are voluntary include walking, rearing, jumping and postural change. The automatic activities are associated with LIA and voluntary activities are associated with RSA. The distinguishing features of automatic activities are that they are relatively stereotyped, usually accompany only one motive state and are difficult to condition to other motive states. In contrast, voluntary motor patterns are flexible, can occur during a number of motive states and are readily conditioned to various motive states. The features of automatic activities suggest that they do not require extensive

forebrain circuits while more voluntary patterns do require forebrain participation. For example, forebrain mechanisms would be active to guide a rat through a complex maze to water but they would not be required once water lapping began. The occurrence of LIA during immobility, as well as automatic movements, is consistent with this interpretation since it would be expected that forebrain circuits would not be activated while an animal is sitting still or resting.

Support for the idea that hippocampal RSA is a correlate of certain movements has been provided by studies showing that RSA is positively correlated with the amount of movement required for a conditioned response (Dalton and Black, 1968). In the latter study dogs were operantly conditioned to press a pedal in the presence of one conditioned stimulus and refrain from pressing in the presence of a second conditioned stimulus. The animals showed more RSA during pedal pushing than when they did not push the pedal. Thus, RSA was associated with the motor response and not the activity of discrimination. It has also been shown that movement can be conditioned by reinforcing the production of RSA. Black, Young and Batenchuck (1970) trained dogs to change the electrical activity in their hippocampus in order to avoid electric shock. One group was reinforced for making RSA and the other group for not making RSA. During training the animals were paralyzed with gallamine triethiodide but when the animals were presented with the discriminative stimulus following recovery the first group produced more RSA and more skeletal responses than the second. This study suggests that skeletal movement can be conditioned in paralyzed animals by reinforcing the occurrence of RSA.

A number of observations are not consistent with the generalization that RSA is related to voluntary movement. For example, movements which should be accompanied by RSA have been reported to be accompanied by a desynchronized EEG. Thus, cats performing a learned response have been found to have a desynchronized hippocampus once the response is well learned (Grastyán, Lissák, Madarász and Donhoffer, 1959) and rats have been observed to have well developed RSA during early exploration while during later exploratory periods the hippocampus is desynchronized (Routtenberg, 1968). In addition, rats jumping around in a box in response to initial presentation of foot shock have been observed not to have RSA (Pickenhain and Klingberg, 1967). Alternately, well developed RSA has been observed when animals are completely immobile. During periods of paradoxical sleep RSA is well developed while the only movements which occur are periodic twitches (Grastyán and Karmos, 1961; Grastyán, 1959; Jouvet, Michel and Courjon, 1959; Jouvet and Michel, 1960). Periods of well developed RSA have also been observed in cats which are looking at their reflection in a mirror or staring at some object but otherwise are making no overt movement (Brown, 1968; Grastyán, Karmos, Vereczkey and Kellényi, 1966), and in rabbits which are in a state of hypnosis (Klemm, 1966).

Criticisms have been made of the suggestion that LIA is related to automatic movement. For example, Paxinos and Bindra (1970) have argued that the movement patterns associated with activities of grooming and face washing are irregular and that the irregularity of the motor pattern may be reflected in the electrical activity. These authors also suggest that movements of lapping and chewing may be

accompanied by such small RSA that it may not be discernable. Komisaruk (1970) has suggested that RSA may simply be a correlate of vibrissae movements in rats and perhaps also a correlate of sniffing and heart beat. In support of this suggestion he found that RSA and these other variables were frequently in phase and he further argues that "residual" RSA is probably present during automatic activities.

In summary, RSA has been found to be a close correlate of voluntary movements while automatic movements and immobility are related to LIA. These observations suggest that RSA is a sign of fore-brain participation in a motor activity, however, in view of a number of contradictions and exceptions, this idea requires further study before it can be fully accepted.

Objectives of the Research

It has been pointed out in the previous discussion that a major criticism against (1) the idea that RSA is related solely to voluntary movement and, (2) that RSA indicates the hippocampus is activated, is that RSA has been found to disappear from the hippocampus during learning. One possible explanation of this disappearance is that RSA changes during training in some electrode placements, but not in others. An alternate explanation is that there are task differences between those studies which find RSA disappears and those that do not. Therefore, during the present investigation an examination will be made of electrical changes at different electrode points during different learning tasks. It is expected that such an investigation will provide an explanation for the discrepancies present in the literature.

If RSA is in fact a reflection of a functional hippocampus, it may be possible to determine the significance of both frequency and amplitude. It has been suggested that frequency is a sign of attention, and alternately it has been shown that frequency can change with movement. In the following investigation changes in frequency will be compared during initiation of movement, steady performance of a movement, and arrest of movement. These investigations will also be extended to different movement patterns such as walking, trotting and galloping and swimming. It is expected that if RSA is a reflection of movement, there should be changes in RSA patterns during different phases of a response as well as changes in RSA patterns during different patterns of movement.

Although the major research will be conducted with the hooded rat as the primary subject, other species will also be used. The other species will include the mongolian gerbil and the cat. The mongolian gerbil is ideal for the investigation of movement since it is an active and lively subject. Alternately, the cat has been used in many investigations and there are suggestions that differences exist in RSA between species as different as the rat and cat.

GENERAL METHODS

Subjects

The results were obtained from 41 male, adult, hooded rats (Quebec Breeding Farms), 13 male and 4 female mongolian gerbils, and 1 male and 3 female cats (obtained from the animal laboratories, University of Western Ontario). All animals were housed singly in a cage in which food and water were continuously available, unless food deprivation was part of the experimental design.

Surgical and Histological Procedures

Two to four pairs of bipolar electrodes were implanted at various locations in the rats and gerbils and six pairs of electrodes were implanted in the cats. The electrodes were either commercially manufactured (Plastics Products Co., Model 3MS-303) or similar hand-made electrodes. The hand-made electrodes comprised two 0.010 in diameter Nichrome wires twisted together and soldered to Winchester subminature components. Both the commercial and hand-made electrodes were insulated to within 0.5 mm of their tips, the tips were separated by 0.5 to 1.0 mm, and one tip was cut 1.0 mm shorter than the other.

The animals were anaesthetized for surgery with pentobarbital sodium and the electrodes were located using standard stereotaxic procedures. The electrodes were fixed in place with watch screws and dental cement. A Winchester subminiature male connector was soldered

to the head of one of the watch screws fixed in the frontal bone as a ground. In the cats, a single ground lead was connected to several watch screws.

The brain structures sampled included the dorsal hippocampus, neocortex, dorsomedial thalamus, septum and posterior hypothalamus. At the end of the experiments, the animals were perfused with isotonic saline and formalin while under deep anesthesia, and the brains were removed. Coronal brain sections, 40μthick, were cut using a frozen technique. The sections were stained with thionin and mounted for histological examination.

Electrical Recording and Stimulation

After a recovery period of at least two weeks, electroencephalographic (EEG) recordings were taken with a six-channel Grass polygraph, Model VII. Both high and low frequencies were attenuated; the usual half amplitude settings were 1 Hz and 35 or 75 Hz.

Electromyographic recordings (EMG) of vibrissae movements were taken through implanted needle electrodes and implanted bipolar electrodes (Plastics Products Co.) inserted under the skin of the rat or gerbil's snout presumably in, or very near, M. levator labii superioris. Electrocardiograph (EKG) recordings were taken through needle electrodes inserted in the skin on either side of the animal's body. Samples of EEG and EMG were displayed on a dual beam oscilloscope. Half amplitudes for EMG recording were usually 10 and 500 Hz.

Electrical brain stimulation was used to produce hippocampal electrographic seizures (fits). The hippocampus was stimulated through

a standard recording electrode and changes in hippocampal electrical activity resulting from electrical stimulation were recorded from the contralateral hippocampus. Stimulation consisted of 6-12 V, 60 Hz, 0.1 msec duration, rectangular pulses. Each animal was given 3 sec trains of stimulation until afterdischarges appeared in the EEG recording. If stimulation did not produce afterdischarges stimulation intensity was raised by 1 V and the procedure repeated until afterdischarges were produced.

Hypothermia was induced by placing rats with clipped fur in a glass jug 10 in high with a 10 in diameter. The jug was filled to depth of 8 in with 10°C water. A rat was left in the water until it had difficulty keeping its head above the surface. Previous investigations (Whishaw, 1970) had shown that an animal's core temperature at this stage was between 18-22°C. The time required to change core temperature was between 10-15 min. Hyperthermia was induced with a heat lamp. Throughout all procedures core temperature was periodically monitored with a tele-thermometer (Yellow Springs Instrument Co.) through a probe inserted 6.5 cm. Body temperature was written on the chart with the EEG recording s at the appropriate places.

Behaviors were recorded on the polygraph chart using the built in markers and a set of external, manually operated buttons which were connected to unused EEG channels. Various movements were also detected using a capacitance-sensing unit (Electrocraft of Canada Ltd.) similar to the unit described by Griffiths, Chapman and Campbell (1967). In addition to recording movement with the movement detector and manually operated signal markers, pertinent observations were written

on the chart as required.

BEHAVIORAL TESTING

General Behaviors

All animals were observed during spontaneous behavior and a permanent record of EEG activity and concurrent behavior was made. Observations on cats were made in an 18 in wide, 49 in long by 36 in high enclosure with a clear Plexiglas face. Gerbils and rats were observed in a 12 in square box with a clear Plexiglas face. This box was placed on four foam rubber cushions and the movement detector was placed beneath the box. Whenever an animal moved the movement of the box was recorded on the chart through the movement detector (with a latency of less than 10 msec) along with the EEG.

The behaviors observed in cats were resting, orienting, eating moist cat food and drinking milk. During observations of feeding the movement detector was placed beneath the food dish so that feeding was recorded on the chart along with the EEG.

The behaviors observed in the gerbils were general exploration, head movements, sitting still and resting, feeding and drinking, face washing and general grooming, territorial marking, foot stomping and various digging behaviors. Male and female gerbils were also observed together while they engaged in various social behaviors including sniffing, mutual grooming and mating.

The behaviors observed in the rats included walking, head movements, position changes of the body while resting, movements of manipulating a food pellet, drinking water and chewing food, as well as

sleeping. During sleep special observations were made during paradoxical sleep. Whenever movements of vibrissae occurred these were recorded on the chart with a manually operated marker. Observations were also made while rats walked on an open table and when they struggled after being picked up.

Records of vibrissae movements were made in rats and gerbils during walking and sniffing and records of EKG were also made from selected animals during these behaviors. EEG records were taken from rats walking in various states of hypothermia and hyperthermia. Animals were encouraged to walk by tail pinching or prodding.

Behavior and electrical activity of the hippocampus was observed in rats following electrically induced hippocampal seizure activity. Electrical stimulation was applied to one hippocampus while electrical recordings were made from the other. The observations were made of EEG activity during walking which was spontaneous or induced by tail-pinching or prodding. Feeding behavior was also observed in food and water deprived rats following hippocampal seizures and avoidance performance was observed (jumping out of an 11 in box) following seizures. Fits were induced during hypothermia (26-27^{o}C) and hyperthermia (41-42^{o}C) as well as when core temperature was normal.

Wheel running Procedure

The motor driven running wheel was 6 ft in circumference. The back was 1/2 in plywood and the "floor" or running surface was 1/8 in pressboard, 8 in wide. The front of the wheel was 1/8 in transparent Plexiglas with an 8 in hole cut in the center. Animals could be put in the apparatus through the hole, and recording leads could also be

brought out through it. All parts of the wheel except the Plexiglas were painted grey. The central axle was driven by a 1/8 hp motor through a set of pulleys which permitted the wheel to run at speeds of 33, 60, 90 or 144 ft/min.

Fourteen rats were trained to run in the motor driven wheel. The wheel ran at 33 ft/min, and the general plan was for the animals to run for longer and longer durations. On the first day, the animals spent 10 min in the wheel. On the second and third days, they spent 30 min and, on the fourth and fifth day, they spent 60 min in the wheel. The sixth and seventh days constituted a rest period and the major results were obtained on the eighth day, when the animals spent 8 hr in the wheel. For two animals training was continued. These animals spent 4 to 6 hr in the wheel on each of the ninth, tenth and eleventh days and then 8 to 9 hr daily after that until they had each completed 50 hr of wheel running.

Five rats received training in running at different speeds. The rats were placed in the running wheel for 30 to 60 min daily for 30 days and in each daily session the wheel was run at 33, 60 and 90 ft/min. It was found that the rats did not run when the wheel was operating at 144 ft/min. The daily procedure was for the animals to run at one of the three lower speeds for 5 to 10 min, then to allow them a brief (5 to 10 min) rest. After this rest, the speed was changed and this procedure was repeated until the end of each session. Following this 30 day "training period" formal observations were made in a single "test day."

The procedure on the test-day was to place the rats in the apparatus and leave them there for nine test periods. Each test period comprised 2 to 5 min with the wheel running at one of the lower speeds; there were three tests at each speed with a 5 min rest between tests. The speeds were presented in a mixed order. During the test sessions, the wheel was started and when the rats were running smoothly EEG records were taken.

Four gerbils underwent a similar 30 day training period, except that the 144 ft/min speed was also used. On the test day they received two 2 min sessions at each of the four speeds, with a 10 min rest period between sessions. The speeds were presented in a mixed order.

Four of the trained rats would occasionally stop abruptyly in wheel and would then start to run again. In order to examine EEG associated with this stop-start pattern, ten observations were taken from each of these rats while the wheel was turning at 60 ft/min.

Records were also made during the initiation of wheel running in two gerbils at wheel speeds of 33 ft/min and 144 ft/min. Each animal was placed in the wheel while it was stationary. When the animals were motionless, the wheel was started and kept running for 30 sec. This procedure was repeated 20 times. For one animal, the wheel operated at the lower speed for the first 10 times and at the faster speed for the second 10 times; the order of the speeds was reversed for the other animal.

Four rats which had been well trained to run at different wheel speeds were given one session in the wheel during which the

wheel turned at 60 ft/min. While the animals were running the hippocampus contralateral to that from which EEG recordings were obtained was stimulated until hippocampal seizure activity was produced. Changes in behavior and EEG activity were noted prior to the electrical stimulation, during the seizure and following the seizure.

Treadmill Procedure

The treadmill was made of a continuous canvas belt 8 ft long and 19 in wide which ran over two rollers, 2 in in diameter and 18 in long. One roller was connected to a 1/6 hp electric motor so that the treadmill belt moved at 56 ft/min. A floor of 1/2 in plywood was placed under the upper surface of the belt to provide solid footing. The treadmill was enclosed in an unpainted plywood box, 49 in long by 18 in wide and 36 in high, with a transparent Plexiglas front. The top of the box was removable.

Two cats were placed in the apparatus daily for two weeks and the daily sessions were gradually increased from 30 min to 2 hr. They were then placed in the apparatus and required to walk continuously for 8 hr. The other two cats were placed in the apparatus for from 30 min to 2 hr for a few sessions, and electrical activity recorded during observation of behavior.

Bar press Procedure

The bar press stand was a flat-black table 20 in by 14 in, with a 5/8 in square moulding around the edge. A round, white, food cup, of 2 in diameter, was screwed to the table top at the middle of one of the longer sides. A bar assembly (Lehigh Valley Electronics)

with a bar 3/4 in wide was mounted 2 in from the food cup on the left side as an animal faced the cup. Pressing the bar closed a microswitch which operated a pulseformer; each pulse activated a pellet dispenser (Ralph Gerbrands Co., Model D) which delivered a 45 mg pellet (P. J. Noyes Co.) to the food cup through a rubber tube. The pellet dispenser was mounted on a 5 in by 2 1/2 in, black wooden block located at the corner of the table, on the same side as the food cup and to the right as an animal faced the cup. Closing the microswitch in the bar assembly also activated an event marker on the polygraph chart; the event marker thus indicated the duration for which the bar was held down. The movement detector was placed under the table, beneath the food cup, and adjusted so that a pen on the polygraph indicated the proximity of the rat to the food cup. When the rat inserted its head into the cup the pen was deflected downwards; when the rat withdrew its head from the cup, the pen was deflected upwards. Thus, the polygraph recorded the occurrence and duration of each bar press, the retrieval of a pellet from the food dish, as well as the physiological recordings.

Ten rats were trained to bar press for food reward; four rats performed the 8 hr wheel running session prior to bar press training, and for four animals the order was reversed, while two animals which were taught to bar press did not participate in the 8 hr running session.

The 10 animals were maintained on a 22 hr food deprivation schedule. After 14 days, they were placed on the bar press stand for 15 min on each of 10 to 15 days. During these 15 min sessions, leads

were attached and electrical activity recorded continuously. Throughout the 10 to 15 sessions, behavioral observations were recorded on the chart, together with the electrical activity. The behaviors recorded included walking, sitting still, rearing, grooming, head movement, food manipulation and chewing. After each of the last eight sessions, the animals were placed in the motor driven wheel (operated at 33 ft/min) for 1 min, and electrical activity was recorded.

Avoidance Procedure

Jump avoidance Procedure. The jump apparatus was an unpainted plywood box, 12 in by 12 in, with walls 11 in high, mounted on four foam rubber blocks. The floor was a grid of 1/8 in, stainless steel rods set about 1/2 in apart and could be electrified by a Harvard inductorium powered by a 1.5 V dry cell battery. A plywood shelf, 2 1/2 in wide, ran around the outside of the apparatus 1/2 in from the top. The movement detector was placed beneath the shelf and connected to the polygraph so that movements of the box were recorded on the chart. A telescoping insert could be fitted which raised the height of the apparatus to 22 in. Thus, the apparatus could be either 11 in or 22 in high.

The jump procedure required an animal to jump out of the jump apparatus within 20 sec in order to avoid electric shock. On the first day the animals were placed in the apparatus with the grid floor uncharged, and allowed to explore for 10 min. After the 10 min exploratory period the animals were removed from the apparatus, replaced on the grid floor and, 20 sec later, continuous shock was administered

until the animals jumped out of the apparatus onto the platform which ran around the outside. After an intertrial interval of 1 min, the animals were again placed on the grid floor and the procedure was continued for 20 trials. Using the same procedure, with the omission of the 10 min exploratory period beforehand, 20 trials were administered daily for three additional days. On the fifth day, after 80 trials, each animal received 30 trials using the same procedure, with the recording leads attached. The results were computed from the first 20 trials in which the movement detector indicated clearly the initiation of the jump avoidance. Initially, seven rats were trained on the jump avoidance in the 11 in box.

Four additional rats were trained to jump both 11 in and 22 in. The first four days of the jump procedure described above was used, except that it was continued for nine daily sessions of 20 trials each and two animals were required to jump 11 in and two were required to jump 22 in. Then the whole nine day procedure was reversed for the animals. Recording leads were attached for the last five daily sessions in each case and the results were computed from the first 10 trials each day in which the movement detector indicated clearly the initiation of the jump avoidance. Thus, data was obtained from 50 jumps at each of the two heights for each animal.

The precise time at which the rats jumped out of the box was checked in one rat by placing a second movement detector beneath a bar at the top of the box to indicate when the rat landed on the platform. Records of when the rat left the grids were also made using a voltage divider circuit which passed a weak current through the grid and the

rat's feet. To determine whether jumping out of the box produced an artifact which could distort the EEG recording, records were taken across 10K and 100K resistors which were attached to the rat's head.

Running avoidance Procedure. The one way avoidance apparatus was a two compartment box, 36 in by 10 in and 18 in high, with a black "start" compartment, 8 in long and a white runway, 28 in long. The floor was a grid of 1/8 in stainless steel rods set about 1/2 in apart and could be electrified by a Harvard inductorium powered by a 1.5 V dry cell battery. A piece of white cardboard, 10 in by 4 in, was placed at the end of the runway furthest from the black start compartment; thus there was a 4 in "safe" area at the end of the runway. A door, hinged on the side of the apparatus, separated the start compartment from the runway. The movement detector was set below the door and detected the first movement made by the animal after the door was opened manually.

Seven animals were trained in the one way avoidance. The avoidance training procedure was the same as the jump procedure except that the animals had to run to the safe area within 5 sec after the door opened in order to avoid electric shock.

Swimming Procedure

The swimming apparatus was a water tight tank, 48 in by 6 in and 12 in high, which was filled with water to a depth of 9 in. The tank was made of 1/2 in plywood painted grey, except that one of the longer sides was made of transparent Plexiglas. At one end of the tank there was a piece of wire mesh which led from the water onto a platform.

Seven rats were trained to swim. For swimming the water temperature was maintained at 38°C. The animals were placed in the water at the end of the tank opposite the wire mesh. One minute after they climbed onto the platform they were replaced at the starting point. Ten such trials were administered daily for four days. On the fifth day each animal received 20 trials, using the same procedure, with the recording leads attached. During these sessions observations were made on the locomotor patterns of the animals. In addition, following testing, additional trials were administered during which records of vibrissae movements were made through EMG electrodes implanted in the animal's snout.

Classical Conditioning Procedure

The test chamber was the unpainted box used for the jump experiments (see above). The top consisted of a piece of Plexiglas with a hole, 3 in in diameter, through which the recording leads could be taken. An audio oscillator (Hewlett-Packard, Model 202C) was connected through a Hunter Decade Interval Timer and an impedance matching transformer to a small speaker on one side of the box. With this system, 5 sec, 750 Hz tones of 75 db could be presented. The movement detector recorded movements of the animal and a small mirror placed above the box allowed the observation of the animal. The oscillator and movement-detector systems, as well as manually operated signal markers, were connected to the polygraph so that the chart simultaneously displayed bioelectrical activity, presentation and duration of tones, administration and duration of foot shock, and certain behavioral observations.

Eight naive rats underwent three tests, conducted in order, following two daily adaption sessions of 2 hr each during which exploration of apparatus was permitted. For the first test, an animal was placed in the apparatus for 30 min and tones were presented at intervals of about 1 min. The first test continued for five days.

For the second test (on the eighth day), an animal was placed in the apparatus for 20 sec and 20 pulsed foot shocks were administered at 1 sec intervals.

For the third test, the animal was placed in the apparatus for 20 min for five daily sessions; the first session followed directly after the second test. In the first three sessions, 20 daily presentations of the tone were given followed by a brief foot shock at tone offset. In the last two sessions only the tone was presented.

Data Analysis

Initially, all records were analysed visually and the recorded behavior compared to the electrical record. Hippocampal electrical activity was classified as either rhythmical slow activity (RSA), large amplitude irregular activity (LIA) or small amplitude irregular activity (SIA). When attempts were made to make counts of the actual occurrence of these waveforms it was found that clear distinctions were sometimes difficult to make. For example if counts of SIA occurrence were being made only very clear occurrences of the pattern (comparable to the illustration in Fig. 17) were included. Similarly, if counts of RSA were made, doubtful cases were excluded. Therefore, estimates of the probability of occurrence of different patterns (as given in Tables 2 and 4) will tend to be low. Activity was classified as RSA if the waves

had a sinusoidal shape, a frequency between 2 and 12 Hz, and an amplitude which was similar from wave to wave. Examples are shown in Fig. 17. Generally, three consecutive waves were required before the sample was classified as RSA. Any waveform which had an amplitude and frequency which were irregular from wave to wave, but nevertheless, had an overall amplitude similar to RSA accompanying walking (in the same animal) was classified as LIA (Fig. 17). Electrical activity which had an amplitude and frequency which were irregular from wave to wave, but an overall amplitude which was as small or smaller than the smallest RSA observed in that animal was classified as SIA. Examples are shown in Fig. 13 and Fig. 16.

The frequency of RSA was measured either by counting the number of waves which occurred within any given time interval or by measuring the period of individual waves with a plastic ruler (mm scale). Mean frequencies were computed from counts of waves and probability distributions of wave frequency were obtained from converted wave periods. The amplitude of RSA was obtained by measuring the amplitude of individual waves.

RESULTS

Electrical Activity and Histological Reconstruction

The pattern of electrical activity recorded from an electrode placed in the hippocampus depends upon two main factors: (1) the behavior of the animal and (2) the anatomical location of the electrode tip.

Depending upon the waking behavior of an animal, rhythmical slow activity (RSA), large amplitude irregular activity (LIA) or small amplitude irregular activity (SIA) could be recorded from a single hippocampal placement. RSA occurred during voluntary movements (walking, running, swimming) and amplitude depended upon the size of body movement; small movements were accompanied by small amplitude RSA and large movements were accompanied by large amplitude RSA. LIA occurred during automatic activities (chewing, face washing) and alert immobility. SIA occurred during active inhibition of movement as in sudden stopping, and on arousal from sleep unaccompanied by movement. These behaviors and associated electrical activity will be fully discussed in succeeding sections.

In terms of the clarity of RSA, electrical activity could also be classified as clear, fast activity mixed with RSA and fast activity. Generally, meaningful distinctions between RSA, LIA or SIA could be made only from placements classified as clear. Examples of these classes of activity are shown in Fig. 1 and Fig. 17. Clear RSA was

defined as RSA which was accompanied by a minimum of fast activity regardless of amplitude. Most sites which yielded this pattern produced waves of high amplitude (greater than 1 mV during running). Mixed activity was RSA accompanied by some fast activity; RSA during large movements (running) could be clearly seen but often no RSA could be seen during smaller movements (bar pressing). Fast activity sites yielded activity faster than 12 Hz with minimal admixture of RSA.

Hippocampal placements of those animals which participated in the bar press task and wheel running task are shown in Fig. 2 and Fig. 3 . From Fig. 2 and Fig. 3 it can be seen that there is a relation between electrode tip placement and the class of electrical activity recorded. Within the dorsal hippocampus clear RSA was recorded from electrodes with the lower tip in the apical dendrites of the pyramidal cells and the upper tip above the cell body of the pyramidal cells in fields CA 1 and CA 2. (Activity in field CA 3 was not sampled.) Fast activity was recorded from electrodes with tips in the dentate gyrus and field CA 4. Mixed activity was recorded from sites between the neocortex and pyramidal cell layer and between the pyramidal cell layer and the dentate gyrus.

Amplitude of RSA depended on the location of the electrode tip as well as behavior. Electrodes with tips across the pyramidal cell dipole of fields CA 1 and CA 2 could yield maximum amplitudes of RSA up to 3 mV, while less optimally placed tips could yield RSA which did not exceed 0.2 mV. Activity at these latter sites was usually mixed. Large amplitude RSA could be recorded from the dentate gyrus-CA 4 area but these sites also gave considerable fast activity.

Fig. 1. Hippocampal EEG in three rats during bar pressing and running in a motor driven wheel. Rat 106, clear RSA (C), electrode tips straddling CA 1 pyramidal cells; rat 119, mixed RSA and fast activity (M), electrode tips between CA 1-2 and dentate gyrus; rat 126, fast activity (F), electrode tips in dentate gyrus-CA 4 area. In rat 106 RSA is well defined during both wheel running and bar pressing. Note the break in RSA during pauses in running and LIA during chewing. In the remaining rats RSA is increasingly obscured by fast activity, especially during bar pressing.

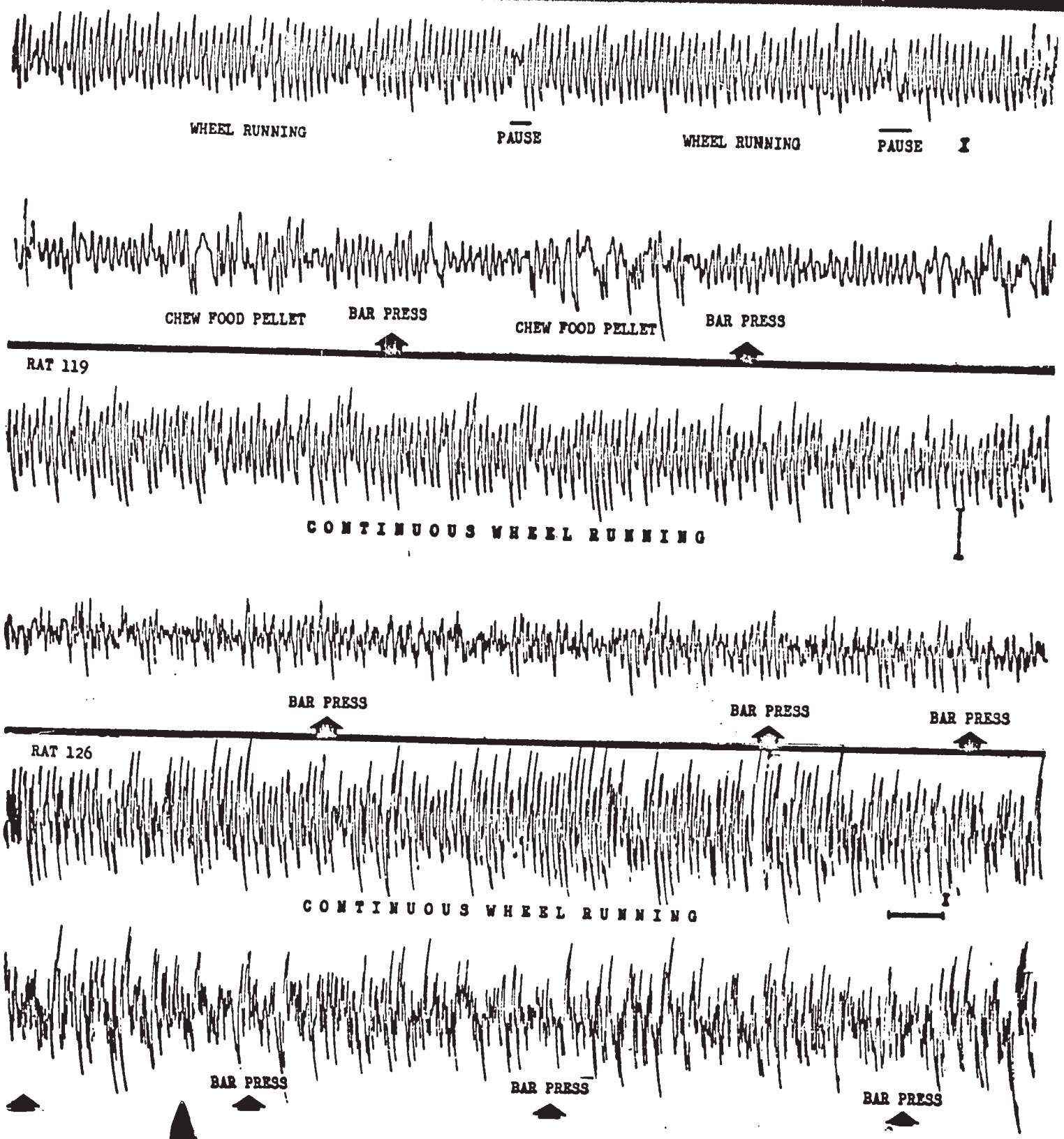
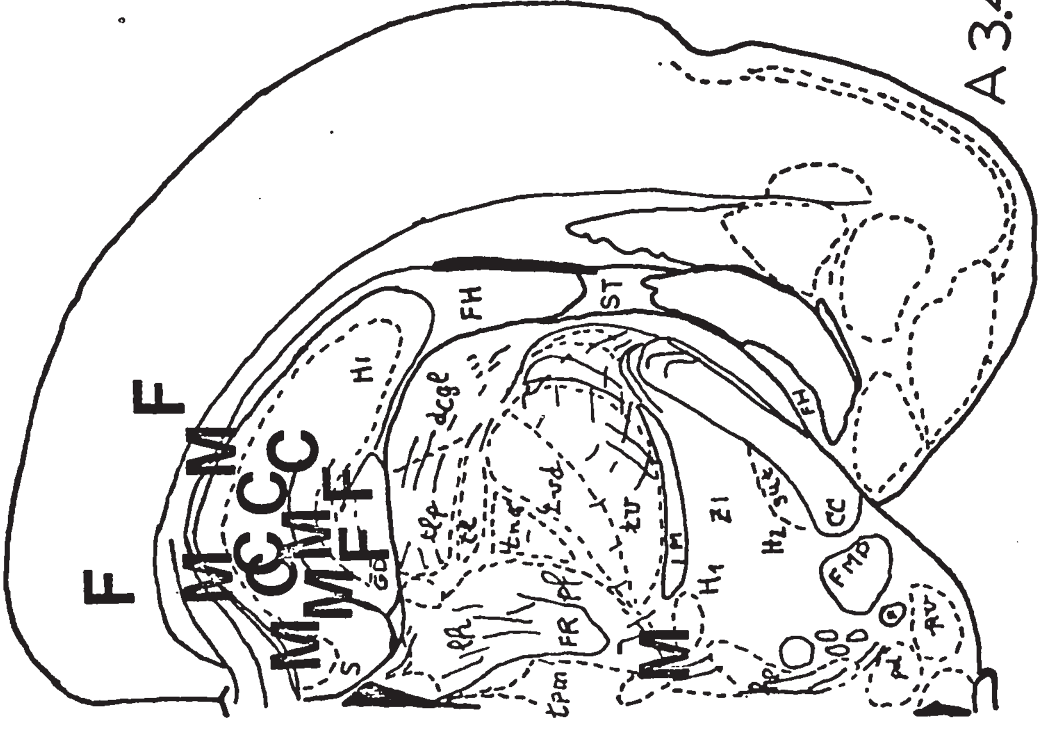
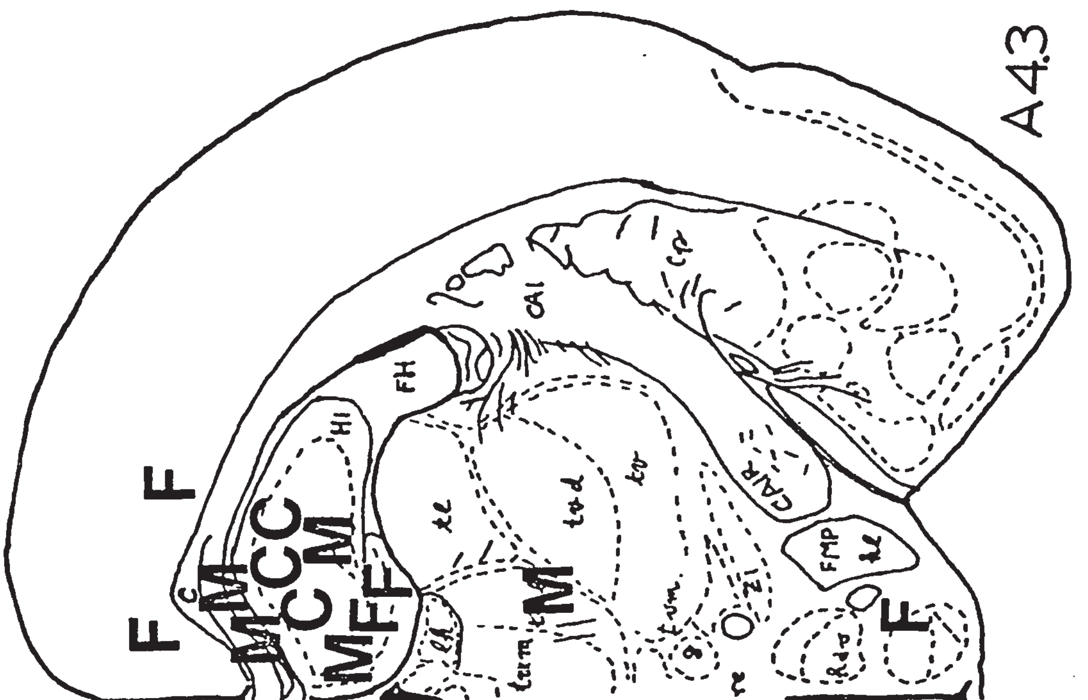

WHEEL RUNNING
PAUSE
WHEEL RUNNING
PAUSE
CHEW FOOD PELLET
BAR PRESS
CHEW FOOD PELLET
BAR PRESS
RAT 119
CONTINUOUS WHEEL RUNNING
BAR PRESS
BAR PRESS
BAR PRESS
RAT 126
CONTINUOUS WHEEL RUNNING
BAR PRESS
BAR PRESS
BAR PRESS

Fig. 2. Coronal sections showing the location of electrode tips in 12 rats from the wheel running and the bar pressing experiments. Numbers in the right hand corner indicate the level in standard A-P stereotaxic co-ordinates. Electrode tips from both sides of the hippocampus are shown on the same side. The placement of the lower electrode tip is shown for each electrode by the letter (the higher tip was about 1 mm dorsal to this). C, RSA accompanied by minimal fast activity; M, RSA accompanied by fast activity; F, mainly fast activity. (Hi - hippocampus; GD - dentate gyrus; S - subiculum). Drawing after König and Klippel (1963). Numbers refer to auterior-posterior coordinates of coronal sections.

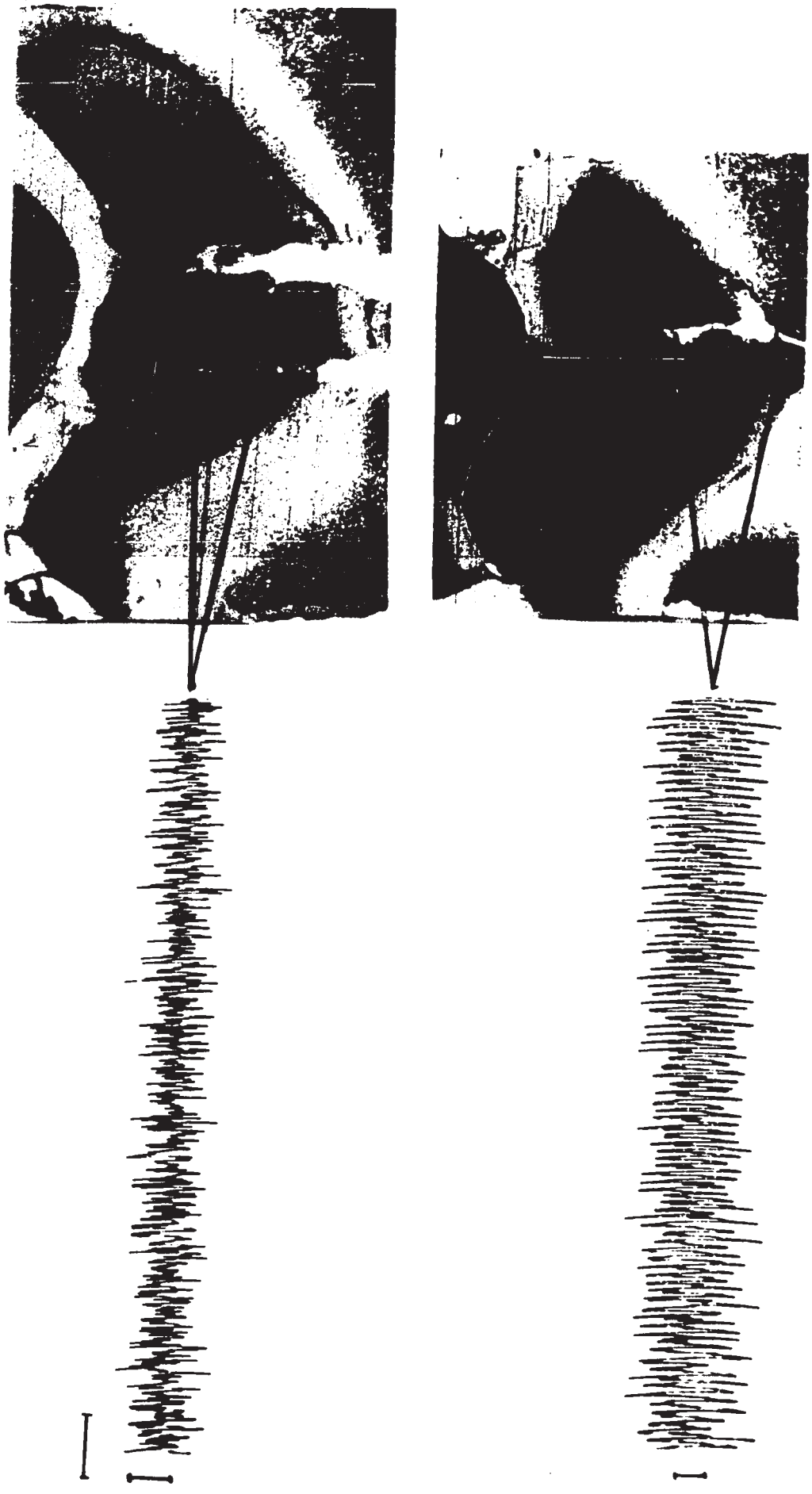


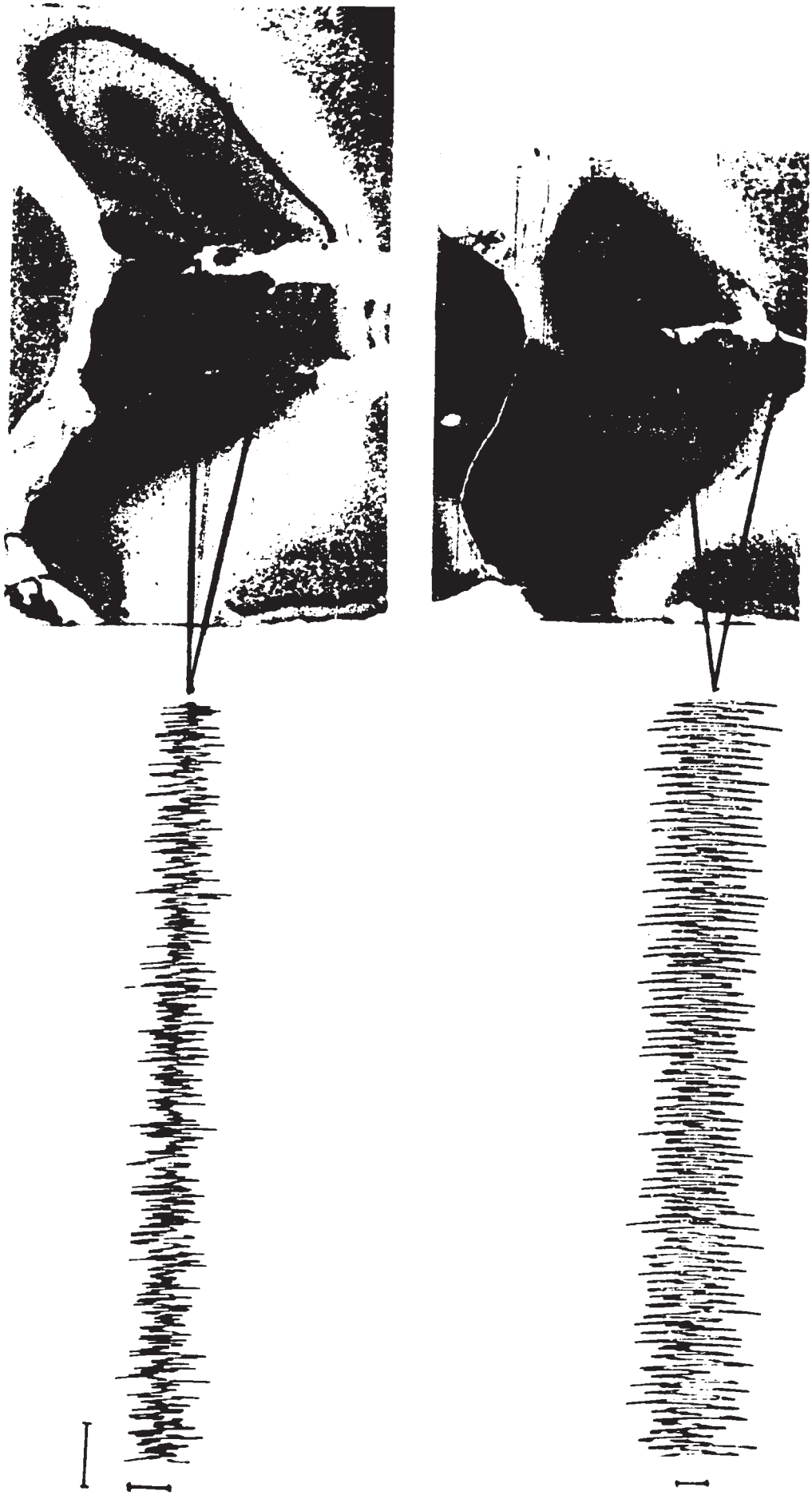
A 3.4



A4.3

Fig. 3. Photomicrograph showing placement of electrode tips in the hippocampus and accompanying EEG. Clear (top) RSA and fast (bottom) hippocampal electrical activity recorded during continuous wheel running. One half amplitude filters; 1 and 75 Hz. Cal: 500 MV and 1 sec.





RSA was not recorded from electrodes with tips in the neocortex, septum or lateral hypothalamus; these sites yielded fast activity. RSA was recorded from two placements in the dorsomedial thalamus and two in the posterior hypothalamus but this activity was of much smaller amplitude than hippocampal RSA.

The type of electrical activity recorded from placements shown in Fig. 1 and 2 was obtained from 12 rats in which detailed comparisons of electrical activity were made during bar pressing and wheel running. This relation was also observed in the rats, cats and gerbils used in other experiments. In all, 29 rats, 11 gerbils and 2 cats were examined which had clear RSA. The remaining animals had placements which yielded mixed or fast activity.

General Observations:

The Relation of Hippocampal EEG to Behavior

The behaviors examined in the following experiments include many which occur spontaneously (without deliberate training) by animals alone, or in pairs, and others which occur in conventional tests of learning, such as bar pressing or shock avoidance. In all situations hippocampal activity was found to be related to the movement occurring at the moment; whether a movement was performed "spontaneously" or as a result of "learning" seemed of minor importance. Therefore, the results have been presented in terms of the relation of hippocampal EEG to movement, first in general terms, then followed by a description of findings in situations which permitted a greater degree of

experimental control over the type of movement performed. In general an observation made in one animal could be easily repeated in the same or in different animals. Therefore, formal results (as distinguished from preliminary findings) are often reported for small numbers of subjects (2-7).

Hippocampal EEG in the Rat

The hippocampal electrical activity in the rat was recorded during a number of waking behaviors as well as when animals were asleep (Fig. 17). Observations on alert animals were made during feeding, grooming and locomotion; sleeping animals were observed during both slow wave sleep and paradoxical sleep.

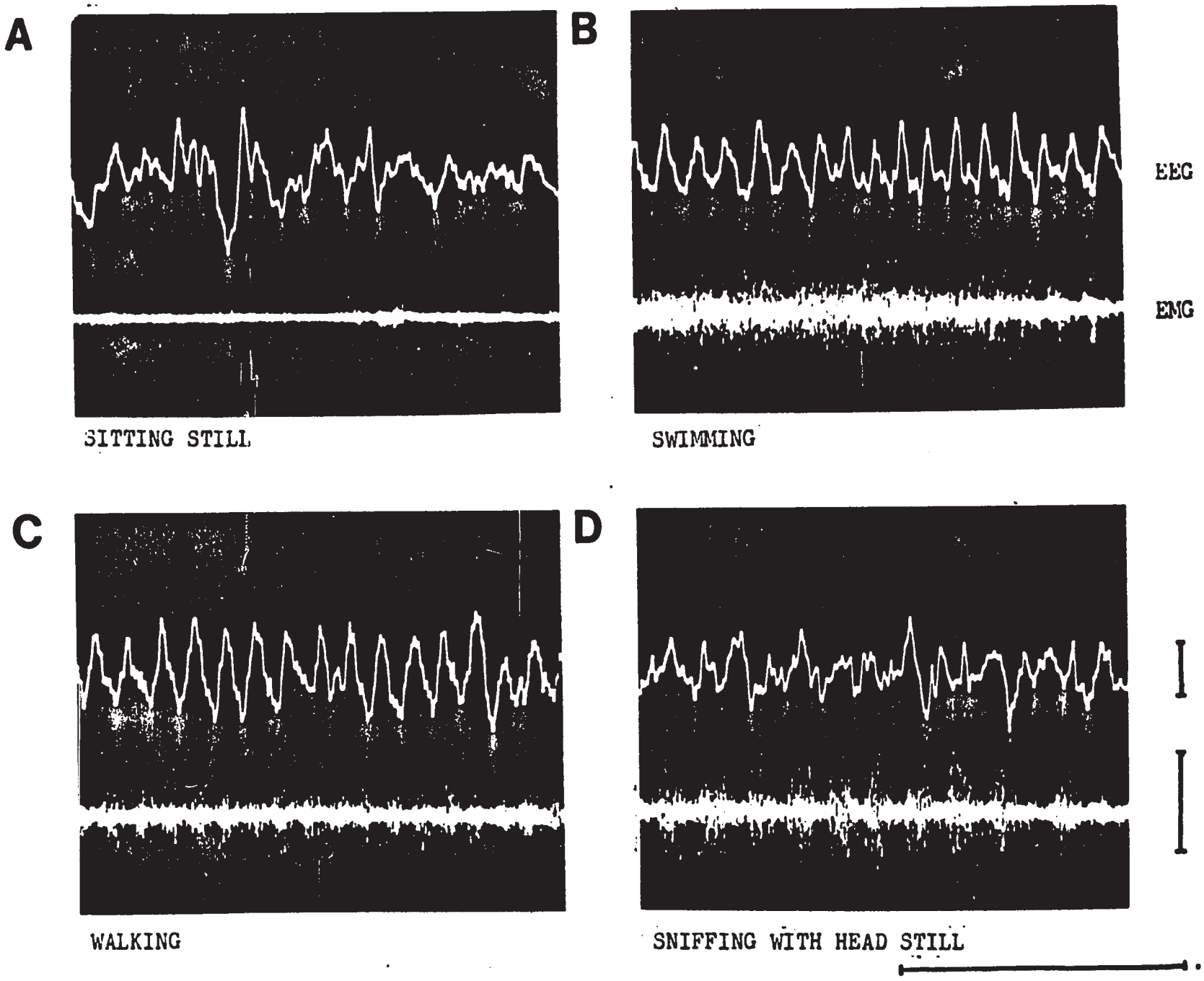
EEG During Waking Behaviors. RSA was found to occur during all forms of locomotion including jumping, running, walking and swimming. RSA was also recorded when animals struggled after being picked up, made small head movements, moved when resting, made postural changes when grooming and when they reared. Heart rate, sniffing and vibrissae movements have been reported to occur in phase with RSA (Komisaruk, 1970), thus heart rate was recorded from four rats as they engaged in a number of behaviors. Heart rate was in phase with RSA on occasion, however, equally often it was not. During wheel running in one rat, for example, heart rate was 7 b/sec during initial running and increased to 8 b/sec as the rat continued to run. RSA remained constant at 7.5 Hz. Vibrissae movements were recorded from four rats during a number of behaviors. Both the EMG and EEG were displayed on oscilloscope and samples were photographed. When rats were sitting still no

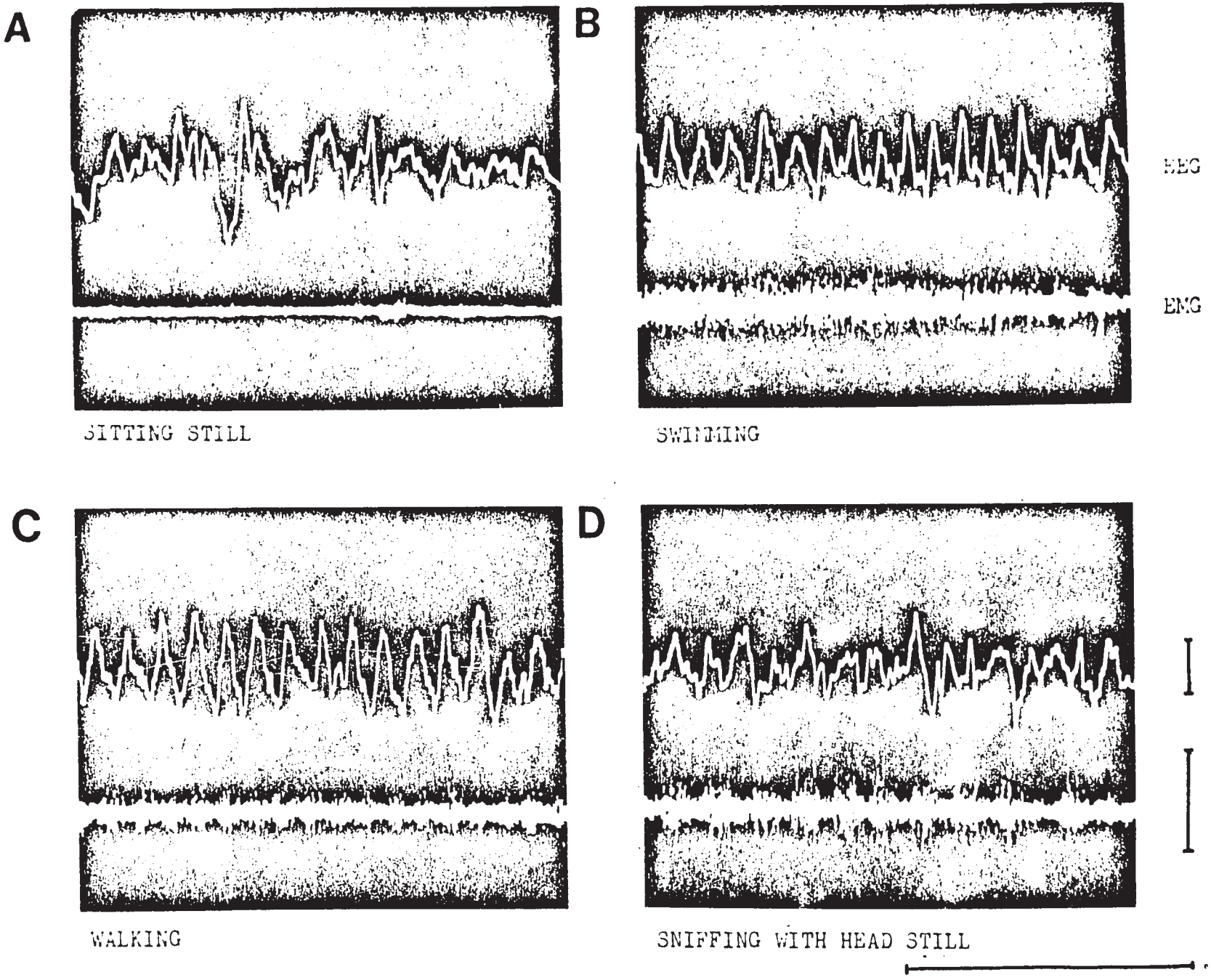
EMG or RSA was recorded (Fig. 4A). When rats were swimming no vibrissae movements were observed and EMG obtained indicated no rhythmical activity indicative of vibrissae movement (Fig. 4B). When animals were walking EMG indicative of vibrissae movements was recorded but was not necessarily in phase with RSA (Fig. 4C). Finally, when rats were sniffing without making other movements EMG activity from the vibrissae was recorded while the hippocampal EEG showed LIA (Fig. 4D).

During feeding, LIA was observed during chewing and lapping water provided other movements of the body did not occur at the same time. LIA was also recorded during face washing and body grooming provided that changes in body posture did not occur simultaneously. LIA was nearly always recorded during alert immobility. Examination of LIA during the different situations in which it was recorded indicated that LIA had much the same appearance whenever it was recorded (see Figs. 1 and 17).

SIA was observed during sessions when rats ran in the motor driven wheel and when they bar pressed for food. (Activity was called SIA if amplitude was as small as or smaller than amplitude of RSA accompanying small head movements.) During these sessions the hippocampal record was at times suddenly reduced in amplitude as shown in Fig. 5. Examination of this suppressed activity at higher gain showed that no RSA occurred for periods which could last as long as one half second; during this period small amplitude waves between 10 and 20 Hz were recorded. This pattern of electrical activity was found only when rats abruptly halted ongoing movement. Two rats which were trained in the wheel, instead of running, jumped across the wheel and

Fig. 4. Relations between hippocampal RSA and vibrissae EMG for four behaviors in one rat. Cal: 1 sec, 500 μV. One half amplitude filters; EEG, 1 and 500 Hz; EMG, 3 Hz and 3 KHz. Electrode placements: hippocampus, field CA 1; EMG electrodes inserted subdermally and bilaterally in the snout.

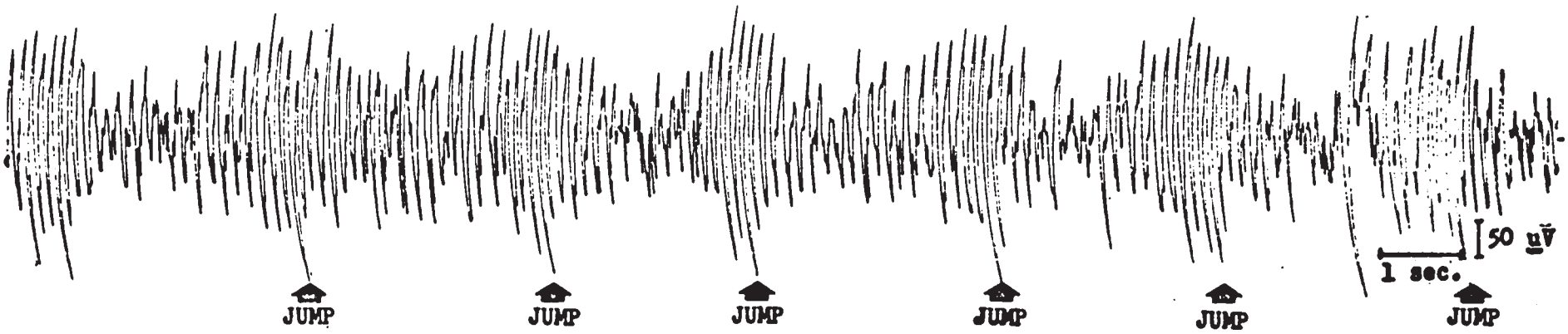

A
B
EEG
EMG
SITTING STILL
SWIMMING
C
D
WALKING
SNIFFING WITH HEAD STILL


A
B
EEG
EMG
SITTING STILL
SWIMMING
C
D
WALKING
SNIFFING WITH HEAD STILL

then rode back in the wheel and jumped again. During the ride back they would crouch, apparently balancing themselves against the wheel's movement, and at this time SIA occurred if the animal was completely immobile or small amplitude RSA occurred if the rat adjusted its posture or moved its head (Fig. 5A). During bar pressing sessions, following a bar press, rats would plunge their heads into the food cup and then suddenly halt the forward movement to seize the pellet of food. SIA was recorded at the point when the rat's head was maximally inserted in the food cup, as was indicated by the movement detector beneath the food cup (Fig. 5B). If the rat moved continuously when retrieving the food pellet SIA was not recorded, rather small amplitude RSA was recorded. During sessions in the running wheel, rats would often pause and remain immobile until the turning wheel forced them to run again. SIA occurred during this period of immobility, however, if the animal raised its head or turned its body RSA was recorded (Fig. 5C). On no occasion was SIA recorded during bar pressing or wheel running in rats with clear RSA unless movement was arrested. A summary of the number of rats and the probability of occurrence of SIA during each of the above behaviors is given in Table 1. The probability of occurrences of RSA during each of the described behaviors in the same recording sessions is also given in Table 1. When RSA occurred on these occasions amplitude was nearly always small as is shown in Fig. 5A. SIA could also be observed when rats were aroused from sleep and this occurrence of the waveform is described below.

Fig. 5. Hippocampal EEG during movement and sudden arrest of movement in the rat. Note: RSA during jump, bar press and wheel running. Suppressed EEG pattern (SIA) occurred: (1) following jump as the rat halted forward motion, (2) following bar press and plunge of head into food cup as rat stopped to seize pellet, (3) during an abrupt halt during wheel running. One half amplitude filters 1 and 35 Hz. Electrode placements: fields CA 1 and CA 2, hippocampus major.

RAT 119: JUMPING IN MOTOR DRIVEN WHEEL



RAT 123: BAR PRESSING FOR FOOD

RAT 121: STOP WHILE RUNNING IN WHEEL

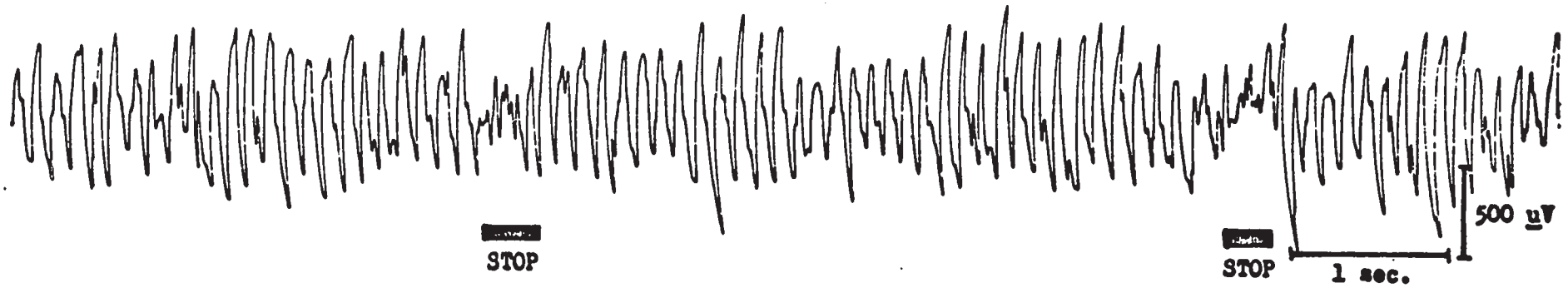


TABLE 1

The Relation Between Arrest of Movement and Hippocampal EEG in Rats

BEHAVIOR	PROBABILITY OF OCCURRENCE			NUMBER OF ANIMALS	NUMBER OF OBSERVATIONS
	RSA	LIA	SIA		
Wheel Run	1.00	0.00	0.00	14	2,429
Pause while wheel running	0.73	0.00	0.27	14	2,429
Jumping	1.00	0.00	0.00	2	914
Pause between Jumps	0.29	0.00	0.71	2	914
Bar Press	1.00	0.00	0.00	6	1233
Pause following bar press	0.36	0.00	0.64	6	1233

Note: In the case of walking an observation period lasted a length of time sufficient to identify the behavior, usually 1-3 seconds.

EEG During Sleep. Electrical activity of the hippocampus was observed during both slow wave and paradoxical sleep. During slow wave sleep hippocampal EEG consisted of alternating periods of high voltage slow irregular activity and high voltage rhythmical "spindles" (about 12 Hz). During slow wave periods of sleep no movements could be observed. Both neocortical and hippocampal EEG was monitored from eight rats during a half hour daily session for five days during which a 5 sec duration tone was presented at intervals of about 1 min. During the early recording sessions the rats were alert but by the later sessions the animals were drowsy or slept during tone presentations.

Changes in hippocampal electrical activity during the 5 sec tone presentation was analyzed in relation to changes in neocortical activity. The results are summarized in Table 2 and examples of EEG changes are shown in Fig. 14. If the animal was alert and low voltage fast activity was already present in the neocortex, RSA appeared in the hippocampus and the movement detector readout indicated that the animal had moved. LIA or, rarely SIA, appeared in the hippocampal records of alert animals when the movement detector remained unchanged. When an animal was resting, high voltage slow activity appeared in the neocortex and hippocampus. If the neocortical activity changed to low voltage fast activity upon presentation of the tone, the hippocampal records showed mostly SIA or RSA of very small amplitude. Because the hippocampal response was usually of such small amplitude, it was frequently difficult to discriminate the RSA from the SIA. In this case, the occurrence of RSA was not related to obvious movement; in

TABLE 2

Changes in Hippocampal and Cortical EEG
During Habituation to a Tone Stimulus

CORTICAL RESPONSE TO TONE ONSET

HIPPOCAMPAL RESPONSE	LVF[1] REMAINS UNCHANGED		HVS[2] CHANGES TO LVF ACTIVITY		HVS REMAINS UNCHANGED	
	PROBABILITY OF OCCURRENCE	BEHAVIORAL RESPONSE	PROBABILITY OF OCCURRENCE	BEHAVIORAL RESPONSE	PROBABILITY OF OCCURRENCE	BEHAVIORAL RESPONSE
RSA	0.71	movement	0.44	movement	0.00	no movement
LIA	0.28	no movement	0.06	no movement	0.00	no movement
SIA	0.02	no movement	0.50	no movement	0.00	no movement
Spindles	0.00	no movement	0.00	no movement	1.00	no movement
	1.01		1.00		1.00	

Note: Results were based on 1,153 observations. Movement refers to the occurrence of visible head, limbs or body movements. [1]LVF - low voltage fast [2]HVS - high voltage slow

about an equal number of instances of RSA and SIA, the movement detector indicated the occurrence of very small movements to tone onset but no movement could be seen by visual observation, except an occasional ear twitch. Finally, in the resting or sleeping animal which was not aroused by tone presentation, the hippocampal record invariably maintained a high amplitude irregular pattern.

Thus, hippocampal desynchronization (that is, SIA) seems to occur only when an animal changes from a resting or sleeping state to an alert state (as indicated by activation of the neocortex) and remains still. If the animal is already alert, RSA occurs with movement and LIA occurs if no movement is made. During initial tone sessions and during the initial tone presentations in later sessions, the animals were usually already alert, and therefore, RSA occurred more frequently as did movements in response to tone presentation. In later sessions the animals tended to rest more and, correspondingly, SIA occurred more frequently.

The electrical activity of the hippocampus was examined in detail in nine rats during paradoxical sleep (as indicated by neocortical desynchronization, hippocampal RSA and maintainance of sleeping posture). During this stage of sleep, which occurred periodically and lasted from 3-15 min, uninterrupted RSA was recorded, while the only movements observed were periodic twitches of the limbs, back, vibrissae, eyes and ears. Examination of RSA frequency during periods of twitches and immobility indicated that RSA frequency was significantly faster during periods of twitching ($N=5$, $t=6.92$, $p < .001$; frequency during twitching 7.72 Hz; during immobility 6.60 Hz).

Results for individual rats are summarized in Table 4, and probability distributions of overall paradoxical sleep RSA and movement periods are shown in Fig. 15. Reference to Table 4 and Figs. 14 and 16, permits comparison of paradoxical sleep RSA to RSA frequencies obtained during waking behaviors. Briefly, RSA frequencies associated with twitching during paradoxical sleep were similar to RSA frequencies recorded during swimming and running and RSA during overall paradoxical sleep was slower than RSA during waking behaviors in which movements occurred.

Hippocampal EEG in the Mongolian Gerbil

Hippocampal EEG recorded from the gerbil was related to behavior in much the same way as in rats. A summary of the number of observations of various behaviors and associated EEG activity is given in Table 2 and examples are shown in Figs. 5 and 6. RSA was recorded when the animals ran, reared, made postural changes and when they engaged in digging behaviors. Gerbils engaged in at least three types of distinct digging behaviors: (1) digging in the sawdust of the observation cage, (2) digging with the front paws against solid objects and (3) digging prior to and following urination and defecation. RSA accompanied all types of digging and amplitude and frequency was generally similar to RSA recorded during locomotion in the observation box. When gerbils dug in sawdust they gathered the sawdust with their front paws and kicked it behind them with their hind paws. The movements made prior to and following urination and defecation were similar. When the animals dug against solid objects, "corner digging,"

they made rapid digging movements with only the front paws and these movements were accompanied by continuous rotary movements of the body to the left and right, while they pushed themselves against the object with their hind feet. RSA was also always recorded when gerbils engaged in territorial marking (Baran and Glickman, 1970), an activity in which they dragged their ventral (glandular) surface as they walked, over small objects placed on the floor of the observation cage.

LIA was recorded during all types of grooming behaviors engaged in by gerbils including face washing, body grooming, fur chewing, tail grooming and genital cleaning. If the animals made other body movements such as position changes during these acts, RSA and not LIA was recorded. LIA was also recorded during acts of conspecific grooming (grooming another gerbil) and during chewing food and lapping water.

Observations of mating were made in four pairs of gerbils (Kuehn and Zucker, 1968) and EEG records were obtained from the male animals. RSA was recorded as the male approached the female and when it mounted the female but did not make thrusting movements. When thrusting movements occurred very small amplitude RSA or SIA was recorded. If intromission occurred the male flipped from the female into a sitting position in which it engaged in genital cleaning. RSA was not frequently observed during this flip and LIA was recorded as genital cleaning began. Male gerbils frequently engaged in foot stomping (Kramis and Routtenberg, 1969) between sessions of mating and this behavior was accompanied by LIA unless the animals also walked at the same time. (Surprisingly, animals were occasionally seen to groom

and foot stomp at the same time.)

SIA was recorded from gerbils during initial greetings when another gerbil was introduced into the observation box. The two animals would approach each other from an angle and then maintain an immobile posture for a few seconds with heads close together. During initial greetings immobility was associated with SIA. Although the number of observations of this effect was not large the effect was quite dramatic. If the introduced animal was left in the box the greeting posture was assumed each time the two animals met. LIA usually occurred during later occasions of greeting.

At times the gerbils sniffed at small objects in the observation cage without making other movements at the same time. During this concentrated sniffing the animal bobbed its head vertically over a point (a very small lateral movement sometimes occurred but was difficult to observe on all occasions), frequently arched its back and then occasionally made territorial marking movements over the point. Concentrated sniffing lasted for a few seconds to as long as 10 sec and was associated with SIA in the hippocampus. Sniffing could also be associated with a lateral head and body movement such as when an animal sniffed along the edge of the box. At these times small amplitude RSA was recorded.

Although LIA and SIA appeared much the same whenever they were recorded, amplitude and frequency of RSA varied with behavior. When a gerbil ran in the motor driven wheel or on an open surface, amplitude of RSA was larger than RSA recorded from the same animal in the observation box. Although both running in a wheel and running in the

TABLE 3

The Relation Between Hippocampal EEG and Movement in the Mongolian Gerbil

BEHAVIOR	NO. BEHAVIOR OCCURRENCES ACCOMPANIED BY DIFFERENT HIPPOCAMPAL EEG PATTERNS			
	RSA	LIA	SIA	TOTAL NO. OBSERVATIONS
Sitting still	0	385	0	385
Face washing	37	212	0	249
Digging in sawdust	192	0	0	192
Corner digging	75	0	0	75
Body grooming (S)	15	67	0	85
Body grooming (M)	87	0	0	87
Scratching	9	63	5	77
Tail grooming	3	10	0	13
Conspecific grooming (S)	7	146	0	153
Conspecific grooming (M)	27	0	0	27
Lapping water	0	47	0	47
Handling food	12	95	0	107
Head movement	224	7	0	231
Walk and Rear	780	0	0	780
Initial greeting	0	2	18	20
Later greeting	0	78	12	90
Mounting	78	0	0	78
Pelvic thrusting	102	16	67	183
Foot stomping (S)	9	35	0	44
Foot stomping (M)	27	0	0	27
Concentrated sniffing (S)	16	19	87	122
Concentrated sniffing (M)	47	0	0	47
Territorial marking	208	0	0	208

S - no other accompanying movement such as postural adjustments or walking

M - act accompanied by postural change or walking

Fig. 6. Hippocampal EEG and behavior in a Mongolian gerbil (#2). Note: (1) the large amplitude and high frequency RSA associated with jumping and the suppressed activity associated with intervening periods of immobility as the animal engaged in a fight with another gerbil (top), (2) the decline in amplitude and frequency of RSA as the animal ran across the observation box and came to a stop (second line), (3) the amplitude of RSA associated with digging in sawdust is lower than the amplitude of RSA associated with jumping or the initiation of running, and (4) the similarity of LIA during eating, drinking, sitting still and face washing. Cal: 1 sec, 500 uV, one half amplitude filters 1 and 35 Hz. Electrode placement: CA 1, hippocampus major.

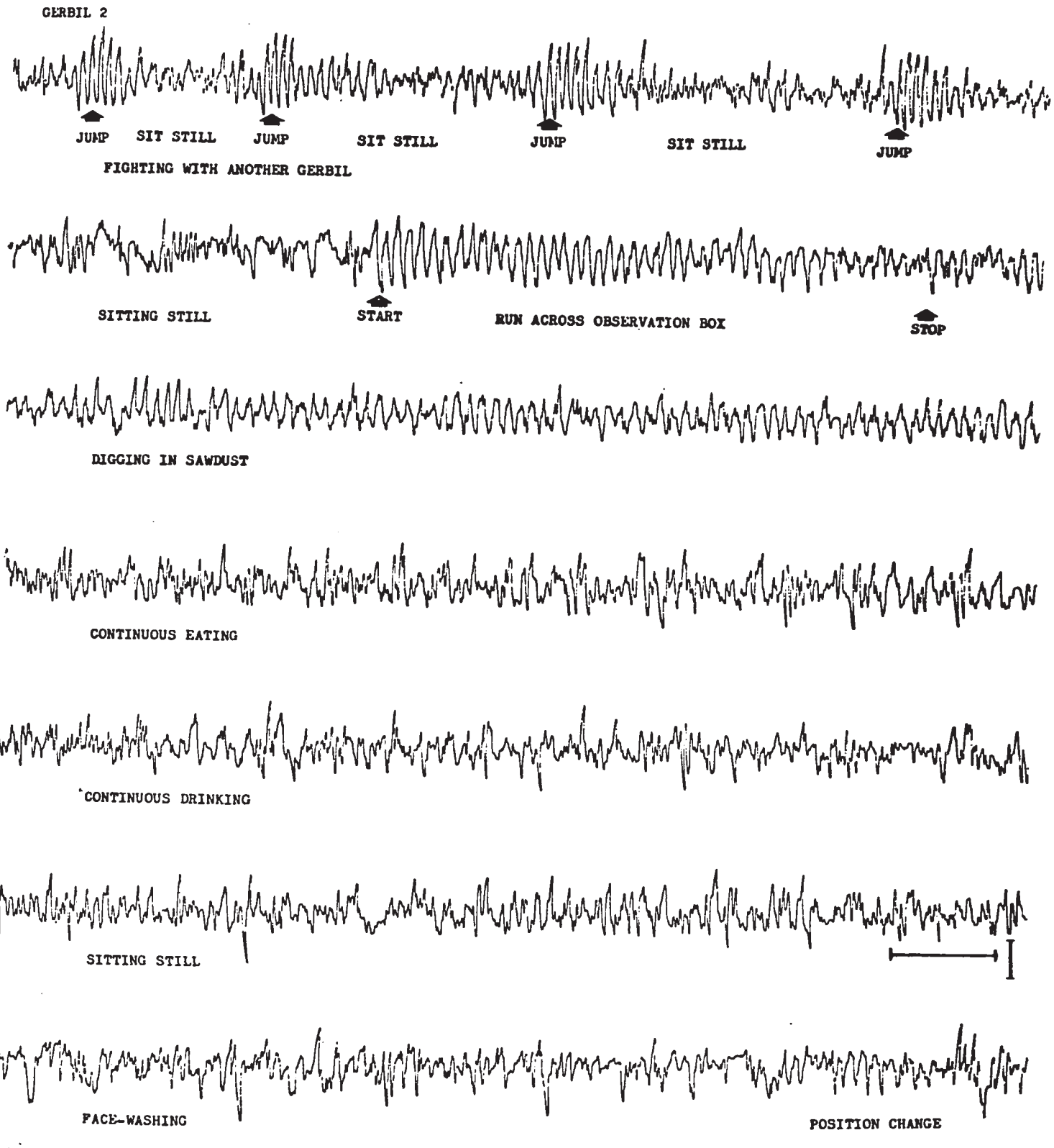
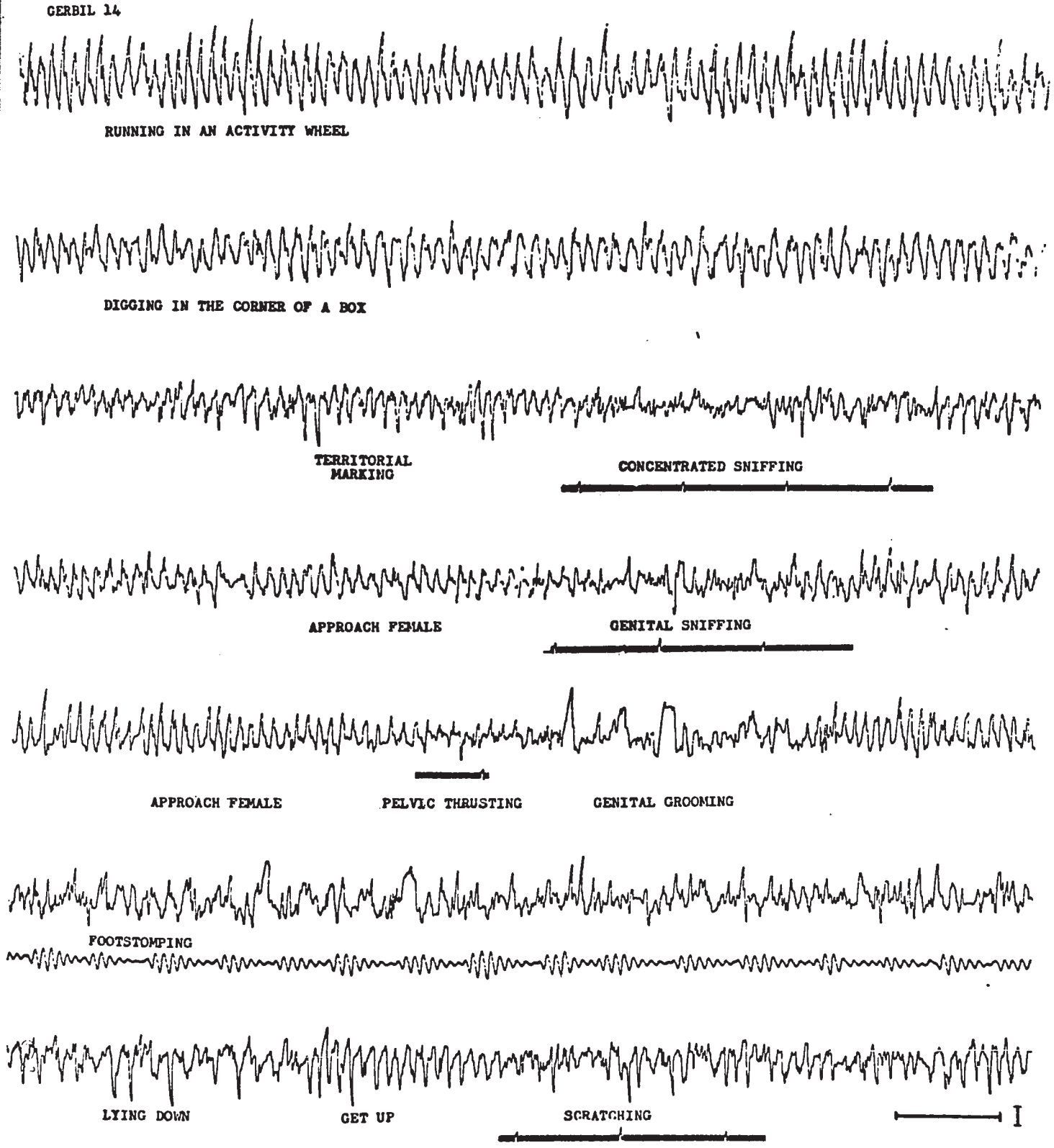

GERBIL 2
JUMP
SIT STILL
JUMP
SIT STILL
JUMP
SIT STILL
JUMP
FIGHTING WITH ANOTHER GERBIL
SITTING STILL
START
RUN ACROSS OBSERVATION BOX
STOP
DIGGING IN SAWDUST
CONTINUOUS EATING
CONTINUOUS DRINKING
SITTING STILL
FACE-WASHING
POSITION CHANGE

Fig. 7. Hippocampal EEG and behavior in a Mongolian gerbil (#14). Note: (1) the differences in amplitude of RSA recorded during wheel running and other behaviors associated with RSA recorded in an observation box, (2) SIA during concentrated sniffing and pelvic thrusting is of smaller amplitude than LIA recorded during genital grooming, foot stomping and scratching. The tracing below the record of foot stomping is the record obtained from the movement detector. Cal: 1 sec, 500 uV, one half amplitude filters 0.3 and 75 Hz. Electrode placement: CA 1, hippocampus major.


GERBIL 14
RUNNING IN AN ACTIVITY WHEEL
DIGGING IN THE CORNER OF A BOX
TERRITORIAL MARKING
CONCENTRATED SNIFFING
APPROACH FEMALE
GENITAL SNIFFING
APPROACH FEMALE
PELVIC THRUSTING
GENITAL GROOMING
FOOTSTOMPING
LYING DOWN
GET UP
SCRATCHING

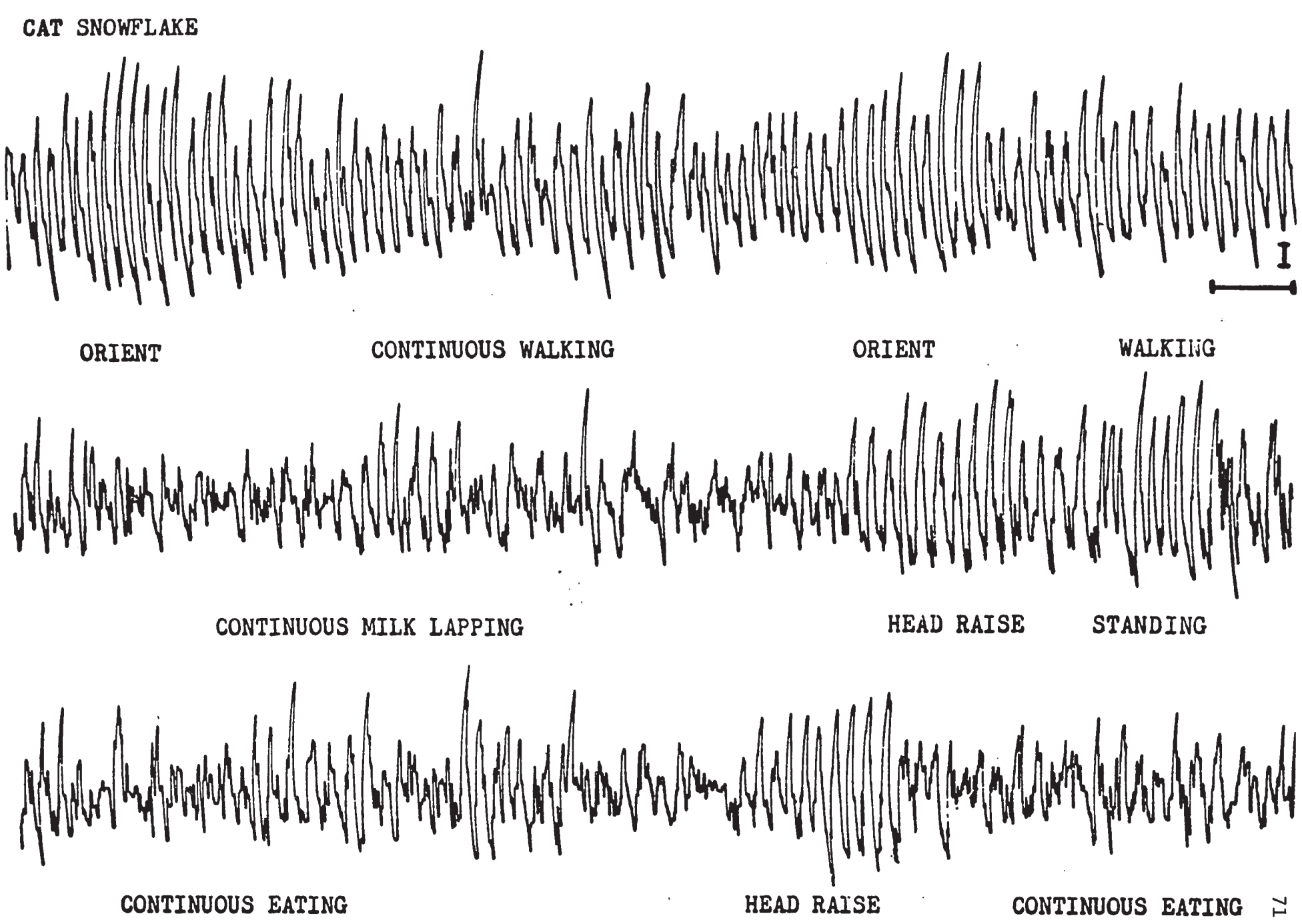
observation box appeared as large movements, the animals usually galloped in long bounds when running in the wheel, while in the observation box the movement pattern seemed to involve a short hopping movement. However, if the animals jumped in the observation box (as could occur during fighting) amplitude of RSA could be quite large. Also, if a resting animal suddenly jumped to its feet and ran across the box amplitude of RSA could be quite large as the animals got up but was smaller as they ran. Amplitude of RSA during territorial marking was usually quite small and as the animals dragged the ventral surface of their body over an object and came to a stop, amplitude of RSA declined steadily and disappeared when the animal stopped. Detailed measurements of RSA frequency were not made from gerbils engaged in spontaneous behaviors but, on casual inspection, these appeared similar to those obtained from rats.

Hippocampal EEG in the Cat

RSA was recorded from at least one electrode placement in each of four cats during changes of posture, walking, head movements and orienting as well as during more formal test situations such as walking in a treadmill, eating cat food and lapping milk. Typical examples of hippocampal EEG activity during these behaviors are shown in Fig. 8.

When the cats walked in the treadmill RSA was recorded continuously during walking and frequency at this time was about 5 Hz. If the cat stretched toward the top of the treadmill as if looking for a route of escape (orienting movement) amplitude of RSA was larger than RSA associated with walking. During the daily sessions of eating cat

Fig. 8. The hippocampal EEG in a cat during walking on a treadmill, orienting, lapping milk and eating cat food. Orienting consisted of a dorsal extension of the head and body toward the top of the treadmill as the cat searched for an exit to escape the treadmill. During milk lapping and eating cat food the animal maintained a crouched position and periodically it would raise its head or stand fully erect on all four feet. Cal: 1 sec, 100 μV, one half amplitude filters, 1 and 35 Hz. Electrode tip location: dorsal hippocampus, field CA 1, the lower tip of the electrode was in the apical dendrite layer of the pyramidal cells.


CAT SNOWFLAKE
1
ORIENT
CONTINUOUS WALKING
ORIENT
WALKING
CONTINUOUS MILK LAPPING
HEAD RAISE
STANDING
CONTINUOUS EATING
HEAD RAISE
CONTINUOUS EATING

food and lapping milk, eating and lapping (as indicated by the movement detector) were found to be associated with LIA. When the animals lifted their heads from the feeding dish or stood up RSA was always recorded. This relation between LIA and eating and lapping was obtained without exception during four days of recording in two food deprived cats and during two weeks of recording in the remaining two animals. Since between 5 and 15 min of feeding was recorded from each animal on each day and no exceptions occurred, this relation was highly significant. However, the relation of hippocampal EEG to movement in the cat is complicated by the fact that RSA often occurs during "fixed staring", a time when the cat seems completely immobile. On many occasions when the cats stared at objects such as the paper moving on the chart or people moving outside the laboratory window no visible movement occurred, nevertheless, well developed RSA was recorded. The relation between fixed staring and RSA was not examined extensively since it has been reported elsewhere (Brown, 1968). EEG recordings were also obtained from cats during grooming behaviors, however, artifacts (which were often rhythmical and in many ways resembled RSA) were not eliminated and so conclusions based upon the data must be limited.

The Relation of RSA Frequency to Behavior

RSA During Steady Locomotion

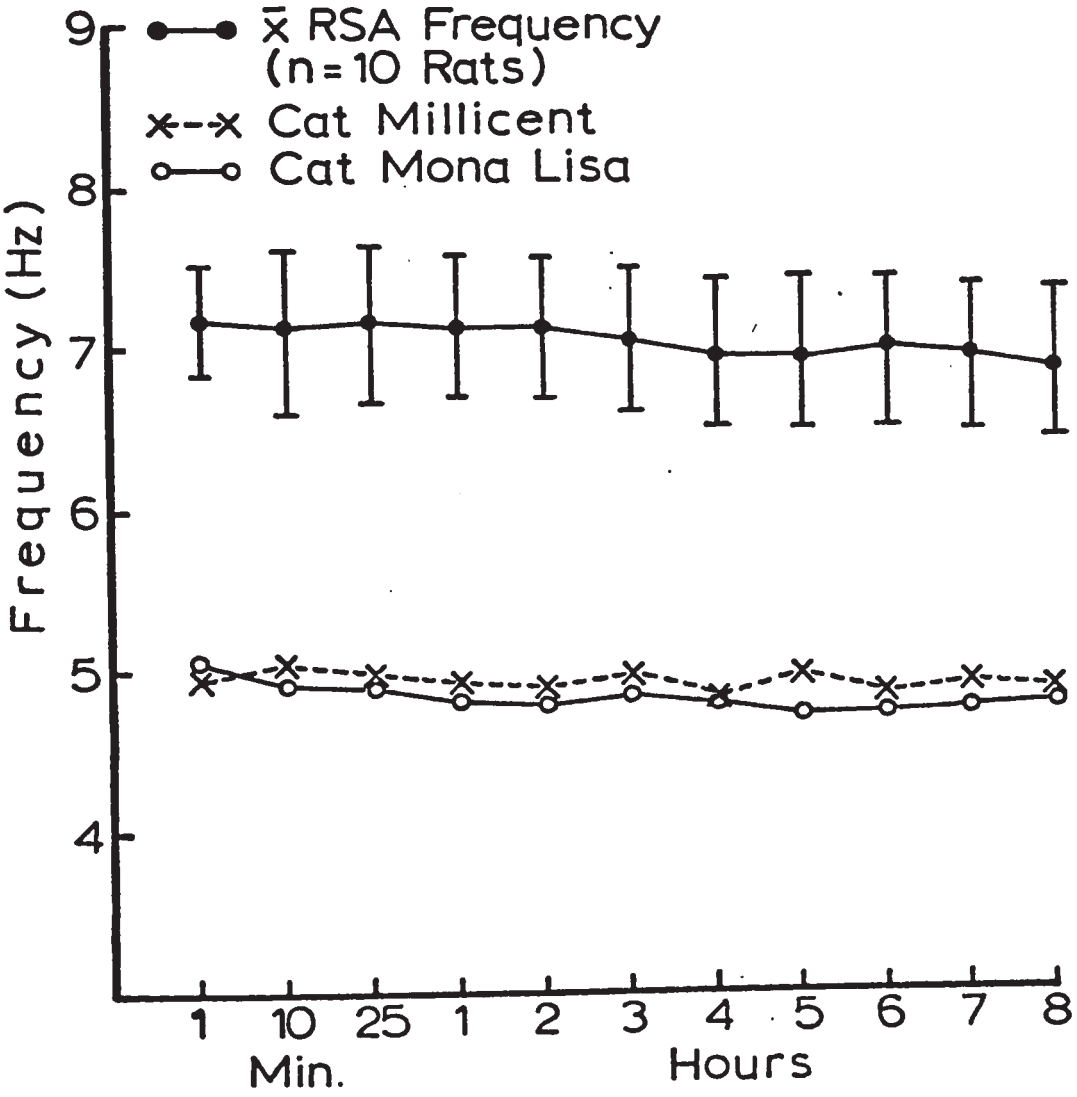
RSA During Prolonged Locomotion in Rats. Recordings of hippocampal activity taken from rats while they walked in the motor driven wheel indicated that RSA was continuously present as long as the animals

moved, even during sessions of walking which lasted 8 hr. Amplitude and regularity of the waveform were unchanged although there was a slight decline in frequency (F=2.85, df=10/90, p <.05) amounting to less than 0.5 Hz. This slight frequency decline appeared due to the increase in the number of "rests" taken by the animals as testing progressed, rather than any intrinsic change in RSA. The mean frequency of RSA associated with walking in the wheel for 10 rats (obtained by counting the number of waves in 1 min samples at various intervals) is summarized in Fig. 8. In the two additional animals which were required to spend 50 hrs in the wheel (distributed over several days) no significant changes in amplitude, frequency or clarity of RSA occurred.

RSA During Prolonged Locomotion in Cats. Hippocampal electrical recordings were taken from four cats as they walked in the treadmill at a speed of 56 ft/min and the results indicated that RSA was continuously present when the animals walked. The mean frequencies of RSA obtained from 1 min samples over an 8 hr test session from two cats is summarized in Fig. 9. Comparison of the frequency of RSA obtained from cats and rats during prolonged walking indicated that the frequency of RSA in rats walking in the wheel was consistently faster (about 2-2.5 Hz) than the frequency of RSA recorded from cats walking in the treadmill.

RSA During Steady Swimming in Rats. Hippocampal RSA was well developed and continuously present during swimming in the seven rats in which observations were made (Fig. 16). Frequency of RSA during steady swimming was constant throughout the response (F=0.79,

Fig. 9. Mean RSA frequency for 10 rats walking in a motor driven wheel at 33 ft/min. Vertical lines indicate standard deviation. Mean RSA frequency is also shown for two cats walking in a treadmill at 56 ft/min. Points indicate mean frequency during 1 min samples of RSA (cats) and mean of ten 1 min samples (rats).


x̄ RSA Frequency
(n=10 Rats)
Cat Millicent
Cat Mona Lisa
Frequency (Hz)
9
8
7
6
5
4
1
10
25
1
2
3
4
5
6
7
8
Min.
Hours

df=20/120, p <.05) with a frequency of about 7.4-7.9 Hz in different rats. The frequency associated with the initiation of swimming was not obtained since the rats were placed in the water manually and were already moving (struggling) when swimming began. Mean frequencies of RSA associated with a steady response of swimming are shown in Fig. 11.

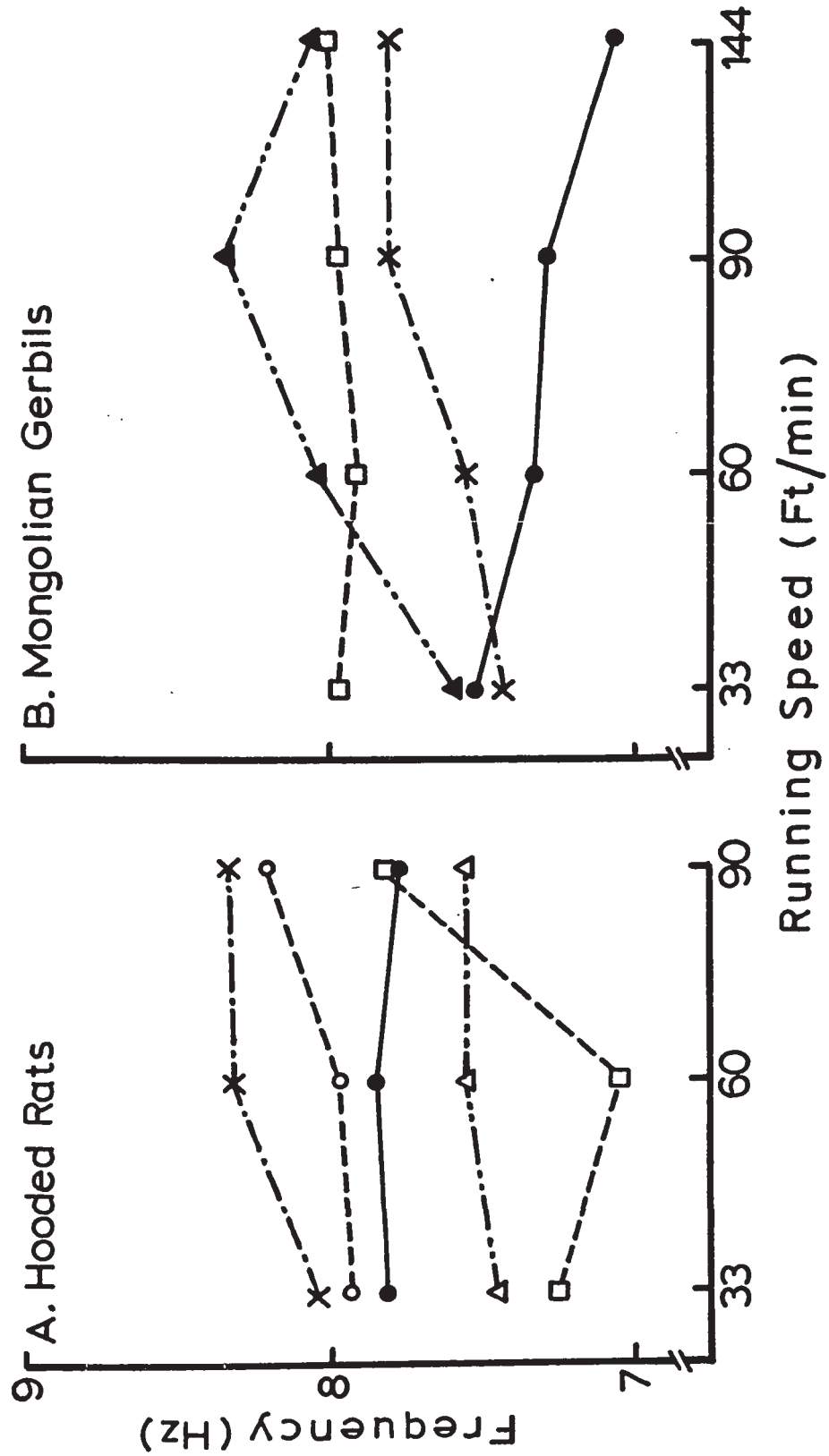
The motor pattern associated with swimming is unlike the motor pattern of walking. During swimming the rat does not use its front limbs to paddle but keeps them tucked beneath its chin while making deep alternate thrusting movements with the hind legs. In addition, an animal's vibrissae do not move during swimming, while during walking they are continuously active.

The Relation of RSA Frequency to Response Speed

The relation between RSA frequency and the speed of running in the motor driven wheel was examined in both rats and gerbils. The results are summarized in Fig. 10. The results from the five rats were based upon a total count of 16,803 waves from the three slower wheel speeds (33, 60 and 90 ft/min). There was no significant difference in RSA during running at different wheel speeds (F=3.81, df=2/8, p <.05). The results for the gerbils were based on a 2 min sample of running at each of four wheel speeds (33, 60, 90 and 144 ft/min) and no significant differences among RSA frequencies were found (F=0.59, df=3/9, p <.05). Thus, RSA frequency did not vary with different running speeds, either in gerbils or in rats.

At different speeds different locomotor patterns occurred. During tests in the wheel, rats walked at 33 ft/min, trotted at 60 ft/min and moved at a fast trot at 90 ft/min. One rat which did run

Fig. 10. The relation between running speed in a motor driven wheel and RSA frequency in five rats and four gerbils. Each point represents the mean frequency of RSA during a 2 min period of steady running.


A. Hooded Rats
B. Mongolian Gerbils
Frequency (Hz)
9
8
7
33
60
90
144
Running Speed (Ft/min)

at 144 ft/min showed a galloping movement and the hippocampal RSA at this speed was not statistically different than during fast trotting at 90 ft/min. The gerbils galloped at all four speeds; only the rapidity of the gallop changed with the running wheel speed. Thus, despite species, locomotor pattern and response speed differences, RSA frequency was similar.

The Relation of RSA Frequency to Movement Initiation

Initiation of movements made by rats such as running and jumping were associated with a rise in frequency of hippocampal RSA but the extent of the rise varied in different cases. Fig. 11 shows shifts in RSA frequency associated with the initiation of jumping and running as shock avoidance responses and with running in a motor driven wheel. The sharpness of the peaks in frequency in Fig. 11 may be related to the method of determining onset of movement rather than any real difference in the behaviors. For example, RSA frequency could be precisely determined during jumping since initiation of the jump was accurately indicated by the movement detector. In the case of one way avoidance, movement initiation was not necessarily as sudden and the movement detector could detect head or body movements before the onset of running. Initiation of wheel running was arbitrarily defined as the first wave following the SIA associated with movement arrest. By using a manually operated movement indicator it was determined that this was approximately the point at which movement initiation occurred. Movement initiation was also examined and frequency of RSA determined for gerbils running at two speeds in the

wheel and for rats jumping 11 in and 22 in. In these cases differences in frequency probably indicate genuine behavioral differences.

RSA Frequency with Movement Initiation in the Rat. When the rats jumped out of the 11 in box, highest frequencies of RSA were concurrent with the jump, with slower RSA frequencies preceding and following the jump (F=18.40, df=20/120, p <.001). Initiation of running in the one way avoidance task was associated with higher frequencies of RSA than occurred prior to and following initiation (F=10.40, df=20/120, p <.001). Initiation of running in the motor driven wheel, after a pause, was associated with higher frequencies of RSA than steady running during which frequency was constant (F=6.86, df=20/60, p <.001). The frequency shifts obtained during steady movement can be seen in Fig. 11 and can be compared to the frequency of RSA found during steady movement (swimming), or wheel running (Figs. 9 and 10).

RSA Frequency with Movement Initiation in the Gerbil. Frequency shifts of RSA were also obtained from gerbils which began running in the motor driven wheel at the moment the wheel was turned on. Significantly higher RSA frequencies were associated with the initiation of running than occurred during steady running (F=26.87, df=20/40, p <.001), and moreover, RSA frequency was significantly higher at the higher wheel speed than the lower wheel speed (Wheel speed x RSA frequency: F=2.73, df=20/40, p <.01). These results are summarized in Fig. 12. Inspection of Fig. 14 indicates that the latter effect was probably due to the differences in RSA frequency during the first half second following movement initiation. It can also be seen

Fig. 11. The relation of RSA frequency to movement initiation in three tasks; (1) jumping 11 in, (2) running in a one way avoidance, and (3) running in a wheel, compared with steady movement during swimming. The "0" and dotted line indicate the first wave period occurring prior to the initiation of movement in jumping and avoidance tasks (as indicated by the movement detector) and the first wave period following a pause during running. The dotted line for swimming indicates that movement initiation occurred prior to the point at which frequency measurements were made. The other numbers indicate the number of waves on which measurements were taken prior to and following movement initiation. The mean frequencies of RSA were obtained by measuring 21 waves associated with each response for each rat on each of 20 trials for jumping, one way avoidance and swimming, and 10 trials for wheel running.

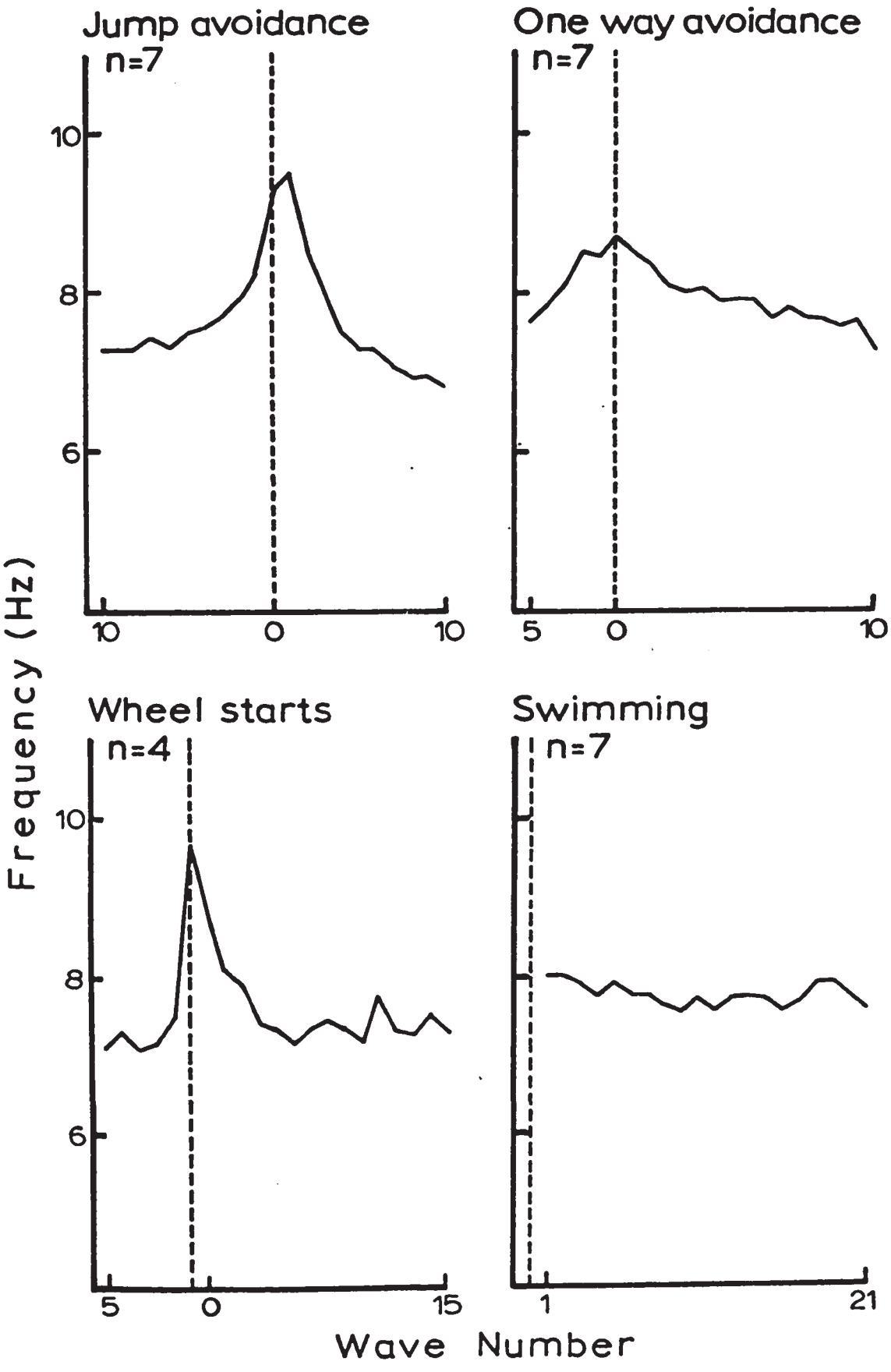
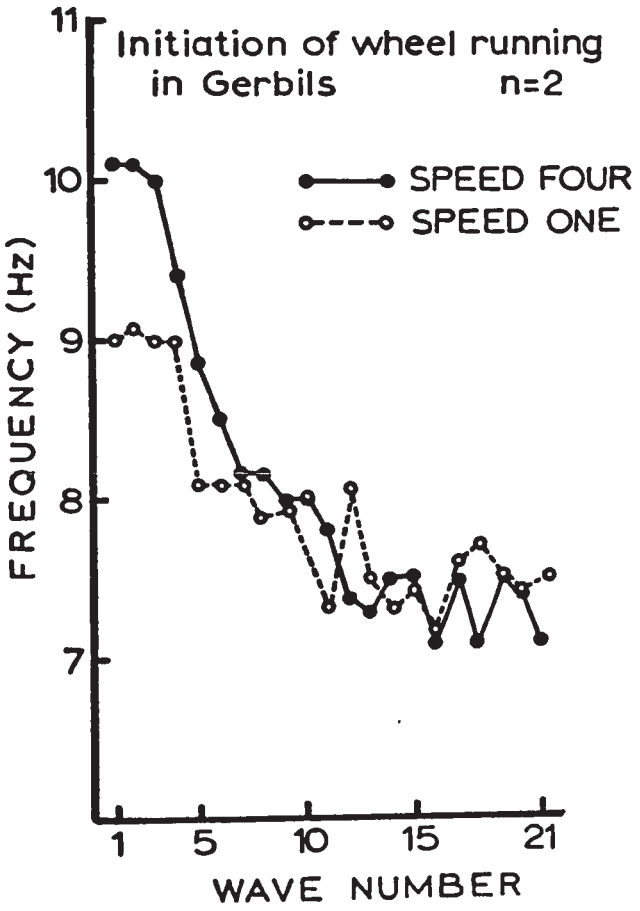

Jump avoidance
n=7
One way avoidance
n=7
Wheel starts
n=4
Swimming
n=7
Frequency (Hz)
10
8
6
10
0
10
5
0
10
5
0
15
1
21
Wave Number

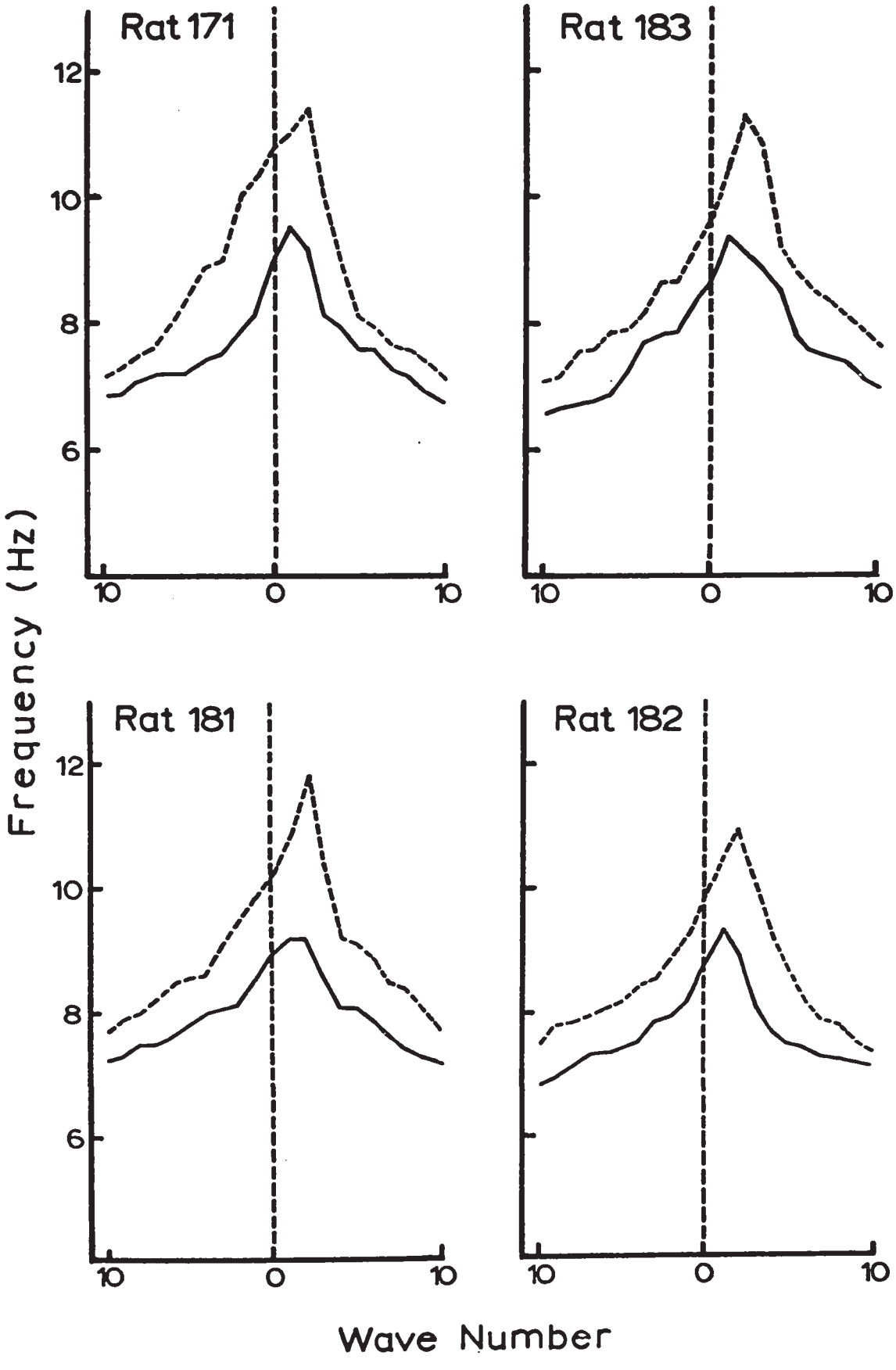
Fig. 12. Mean RSA frequency for initiation of running and steady running in two gerbils at two speeds in a motor driven wheel. Speed "one," 33 ft/min; speed "four," 144 ft/min. Each animal received 10 trials at each wheel speed.


Initiation of wheel running
in Gerbils n=2
SPEED FOUR
SPEED ONE
FREQUENCY (Hz)
11
10
9
8
7
1
5
10
15
21
WAVE NUMBER

in Fig. 12 that RSA frequency during steady running was not related to wheel speed.

RSA Frequency with Initiation of Jumps of 11 and 22 in. When the frequency of RSA was compared for rats jumping out of boxes of different heights (11 in and 22 in), the frequency of RSA was found to be higher during the higher jump (F=42.88, df=1/6, p <.001). These results are summarized in Fig. 13 and an example of hippocampal EEG associated with jumps at the two heights is shown in Fig. 17. RSA frequency also increased with movement initiation and decreased afterwards (F=100.41, df=20/120, p <.001) for both the low and high jump. Finally, there was a significant interaction between this frequency shift and the height of the jump (F=7.08, df=20/120, p <.001). Inspection of Fig. 13 shows that this interaction effect is probably due to the fact that the fastest wave in the 22 in jump occurred two waves after the "0" (wave preceding jump initiation as indicated by the movement detector) while the fastest wave in the 11 in jump occurred only one wave after "0". When rats jumped out of the box, they jumped, caught the edge with their forepaws and then pulled themselves up onto the edge. On the 11 in jump, when a small bar was placed above the edge so that the rats jumped and caught the bar (displacement of which activated a second movement detector) it was found the fastest wave occurred between the time the rats left the grids and caught the bar. On the 22 in jump a circuit was passed through the rats and grid in order to activate a chart marker while the rat was on the grid. Use of this procedure indicated that the rat was still on the grid when the first wave after "0" occurred. It was concluded, therefore, that

Fig. 13. Mean frequencies of hippocampal RSA associated with jumping out of boxes of two different heights in four rats. The dotted curve represents the frequency of RSA associated with jumping out of a box 22 in high and the solid curve represents the frequency of RSA associated with jumping out of a box 11 in high. The "0" dotted line represents the wave period immediately preceding jump initiation as indicated by the movement detector. Each curve represents the mean of 50 trials.


Rat 171
Rat 183
Rat 181
Rat 182
12
10
8
6
10
0
10
Frequency (Hz)
Wave Number

the fastest wave in both the 11 and 22 in jump occurred after a rat left the grid, but before it caught the edge of the box with its forepaws. Recordings across resistors (10 KΩ and 100 KΩ) placed on an animal's head during jumping indicated no significant electrical activity due to movement or other sources of artifact.

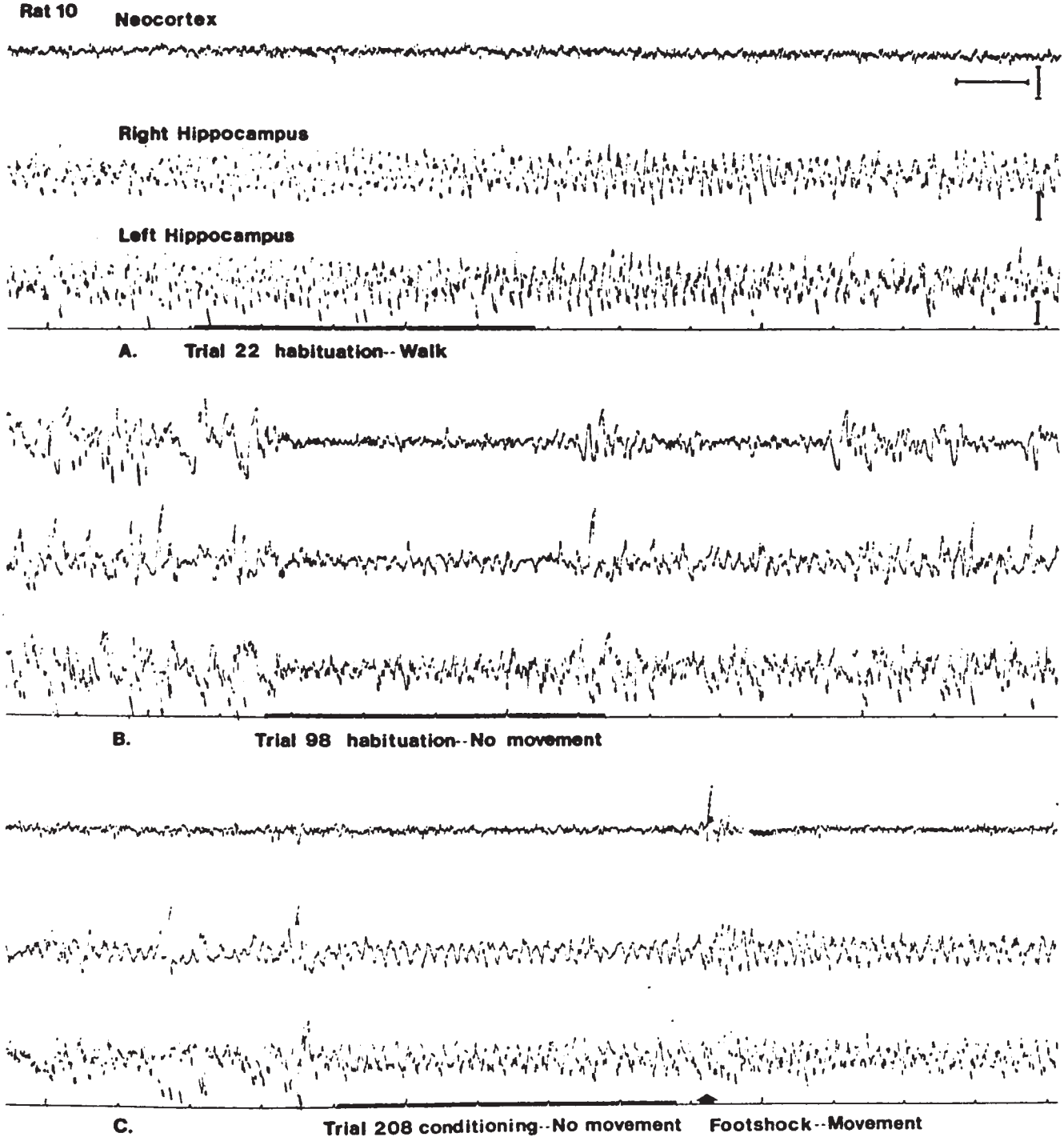
The Relation of RSA Frequency to Immobility

In experiments in which a 5 sec tone was presented to eight rats, three tests were conducted. The results of the first test are reported fully in the section on sleep. Briefly, if an animal moved in response to the tone, RSA occurred, while if the animal was alert but immobile LIA occurred. If an animal was resting and was aroused but did not move SIA occurred with tone onset.

In the second test the animals were presented with 20 brief foot shocks spaced at 1 sec intervals. The response to the foot shocks was vigorous movement and jumping about; the hippocampal records invariably showed RSA similar to walking during this behavior.

In the third test, after several pairings of the tone and foot shock, the animals ran around the box and attempted to escape with tone onset; RSA was clearly present during these activities. As the tone and shock pairings continued, the animals tended to freeze, or remain immobile, with tone onset. In this case, RSA of lower frequency (Mean frequency = 5.87 Hz) appeared in the hippocampal record and this lower frequency RSA continued to appear after foot shock no longer followed the tone. This slow RSA occurred after the first day of conditioning, and by the time tone presentations were

Fig. 14. Changes in neocortical and hippocampal electrical activity are shown during habituation and conditioning to a 5 sec tone (marker): (A) Upon presentation of the tone the rat walks across the observation box, and RSA appears in the hippocampal record. (B) The rat is sleeping. At tone onset the rat wakes up but does not move, and both the hippocampus and neocortex desynchronize. (C) Following many pairings of the tone and foot shock the rat freezes at tone onset, nevertheless, slow RSA appears in the hippocampus. Following the tone the rat moves in response to foot shock and both amplitude and frequency of RSA increase. Cal: 1 sec, 500 u V, half amplitude filters 0.3 and 75 Hz. Electrode placements: Anterior neocortex and hippocampus major, fields CA 1 and CA 2.


Rat 10
Neocortex
Right Hippocampus
Left Hippocampus
A. Trial 22 habituation--Walk
B. Trial 98 habituation--No movement
C. Trial 208 conditioning--No movement Footshock--Movement

given unaccompanied by foot shock the relation was well established. The only observed change which occurred in these later sessions when tone was presented alone was an increase in the regularity of respiration. Examples of change in electrical activity associated with behavioral responses to tone onset are shown in Fig. 14. The frequency histogram for RSA associated with conditioned immobility is shown in Fig. 15. It can be seen from Fig. 15 that the distribution is unimodal with a peak at 6.0 Hz similar to the slower peak in the bimodal frequency histogram for overall paradoxical sleep. Mean RSA frequencies for five rats during conditioned immobility are shown in Table 4 and it can be seen from Table 4 that RSA frequency at this time is far slower than RSA associated with other behaviors.

Summary of the Relation of RSA Frequency to Behavior

A summary of the major relations of frequency of RSA to behavior is given in Fig. 15 and Table 4. Probability distributions of RSA obtained from behaviors involving movement were unimodal with peak frequencies at 7.5 Hz, while the distributions for overall paradoxical sleep is bimodal with a second peak at 6.0 Hz. There were no statistical differences in frequencies of RSA associated with running, swimming and movements occurring during paradoxical sleep but RSA frequencies associated with these movements were higher than RSA frequencies associated with bar pressing in all animals ($U=1$, $p < .05$). The frequency of RSA during overall sessions of paradoxical sleep were statistically lower than frequencies associated with behaviors involving movement ($U=0$, $p < .05$). Finally, frequency of RSA associated with a

Fig. 15. Probability distributions of hippocampal RSA waves for six behaviors. The distributions were compiled from the data summarized in Table 4. Measurements are not necessarily based on the same rats throughout.

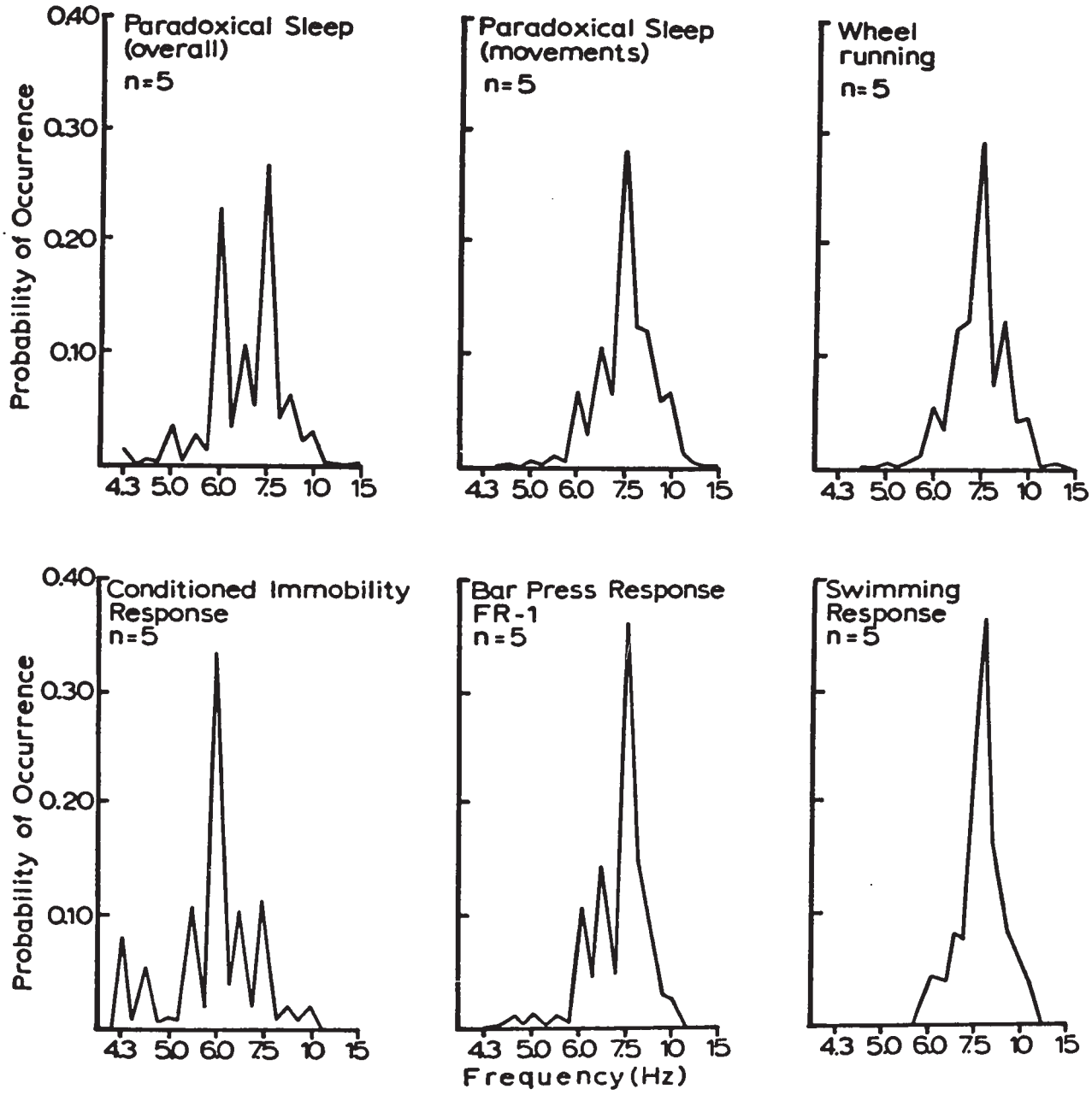

Paradoxical Sleep (overall) n=5
Paradoxical Sleep (movements) n=5
Wheel running n=5
Conditioned Immobility Response n=5
Bar Press Response FR-1 n=5
Swimming Response n=5
Probability of Occurrence
0.40
0.30
0.20
0.10
4.3 5.0 6.0 7.5 10 15
Frequency (Hz)

TABLE 4

The Relation Between RSA Frequency and Behavior

BEHAVIOR	MEAN RSA FREQUENCY	RSA FREQUENCY OF INDIVIDUAL RATS					TOTAL NO. OF PERIODS MEASURED
1. Wheel Running (60 ft/min)	7.7	7.1	7.6	7.8	7.9	8.3	5,519
2. Paradoxical Sleep (movements)	7.6	7.3	7.5	7.7	7.7	7.9	6,536
3. Swimming	7.6	7.4	7.4	7.6	7.6	7.9	2,450
4. Bar Pressing	7.2	7.0	7.1	7.2	7.2	7.4	2,513
5. Paradoxical Sleep (overall)	6.7	6.5	6.7	6.8	6.8	6.8	9,088
6. Conditioned Immobility Response	5.9	5.6	5.6	5.8	6.0	6.0	1,872

1. The mean RSA frequencies for behaviors 1, 2 and 3 were not significantly different ($p>0.05$)
2. The mean RSA frequencies for behaviors 1, 2 and 3 were significantly higher than those for behaviors 4, 5 and 6.
3. The mean frequency for behavior 4 was significantly higher than that for behavior 5.
4. The mean frequency for behavior 5 was significantly higher than that for behavior 6.

conditioned immobility response was lower than frequency recorded during any other behavior ($U=0$, $p < .05$), and the modal frequency (6.0Hz) was the same as the second peak in the overall paradoxical sleep distribution.

Thus, frequency did not vary with response speed during steady locomotion or locomotor pattern (walking, trotting, galloping or swimming). Frequencies did, however, increase during the initiation of movement and the amount of increase depended on the speed of response initiation or height of jump. Species differences were found between cats and rodents but there were no apparent frequency differences between the two rodent species. Finally, frequencies of RSA associated with immobility (periods of no movement during paradoxical sleep and conditioned immobility) are slower than frequencies of RSA associated with behaviors involving movement.

Relation of RSA Amplitude to Behavior

The amplitude of RSA was examined in 10 rats which participated in the bar pressing and wheel running tasks. Amplitude of RSA was also examined in four additional rats over a wider range of behaviors.

RSA Amplitude with Bar Pressing and Wheel Running

Ten rats which had been food deprived were placed on an open stand for daily 15 min sessions until they discovered that a bar located on the stand could be pressed to obtain food. When the animals were initially placed on the stand they engaged in exploratory movements (walking, rearing) during which RSA amplitude was large and

similar in amplitude to RSA recorded from the same animals while they ran in the motor driven wheel (Fig. 1). During a bar press session when the animal's activity changed from exploration to pressing the bar, RSA also changed. At clear RSA sites RSA decreased in amplitude, at mixed sites RSA was often present initially but disappeared as rats learned to bar press. At fast sites RSA could occasionally be seen during exploratory activities but was never observed during bar pressing. At all placements the overall EEG amplitude decreased during bar pressing as compared to exploration and wheel running (Fig. 1). In the 10 animals the mean reduction in amplitude between bar pressing and wheel running was 44% (range: 35-52%). If animals made slow vertical head raises which were also associated with vertical trunk extension, RSA amplitude was as large as RSA found during wheel running (90 such observations were made in six rats).

RSA Amplitude with Other Behaviors

The results of the examination of RSA amplitude changes over a wider range of behaviors in four rats are summarized in Fig. 16 and examples of amplitude differences in one rat are shown in Fig. 17. The results indicated that larger movements of jumping, walking, struggling and swimming had larger RSA amplitudes than smaller movements of the head, or the paws (when handling a food pellet) or the whole body (with position shifts during resting). During paradoxical sleep a wide range of amplitudes of RSA were recorded but during periods of movement amplitudes of RSA were larger than during periods of non-movement.

Fig. 16. The mean amplitude (bars) and ranges (vertical dotted lines) of hippocampal RSA in four rats during nine behaviors. Amplitude measurements were obtained from records for which half amplitude filters had been set at 0.3 and 75 Hz. The amplitude of RSA accompanying each behavior in each rat was determined by measuring 200 individual waves, with the exception of jumping, for which 20 waves occurring at the moment of jumping on each of 20 avoidance trials (as indicated by the movement detector) were measured.

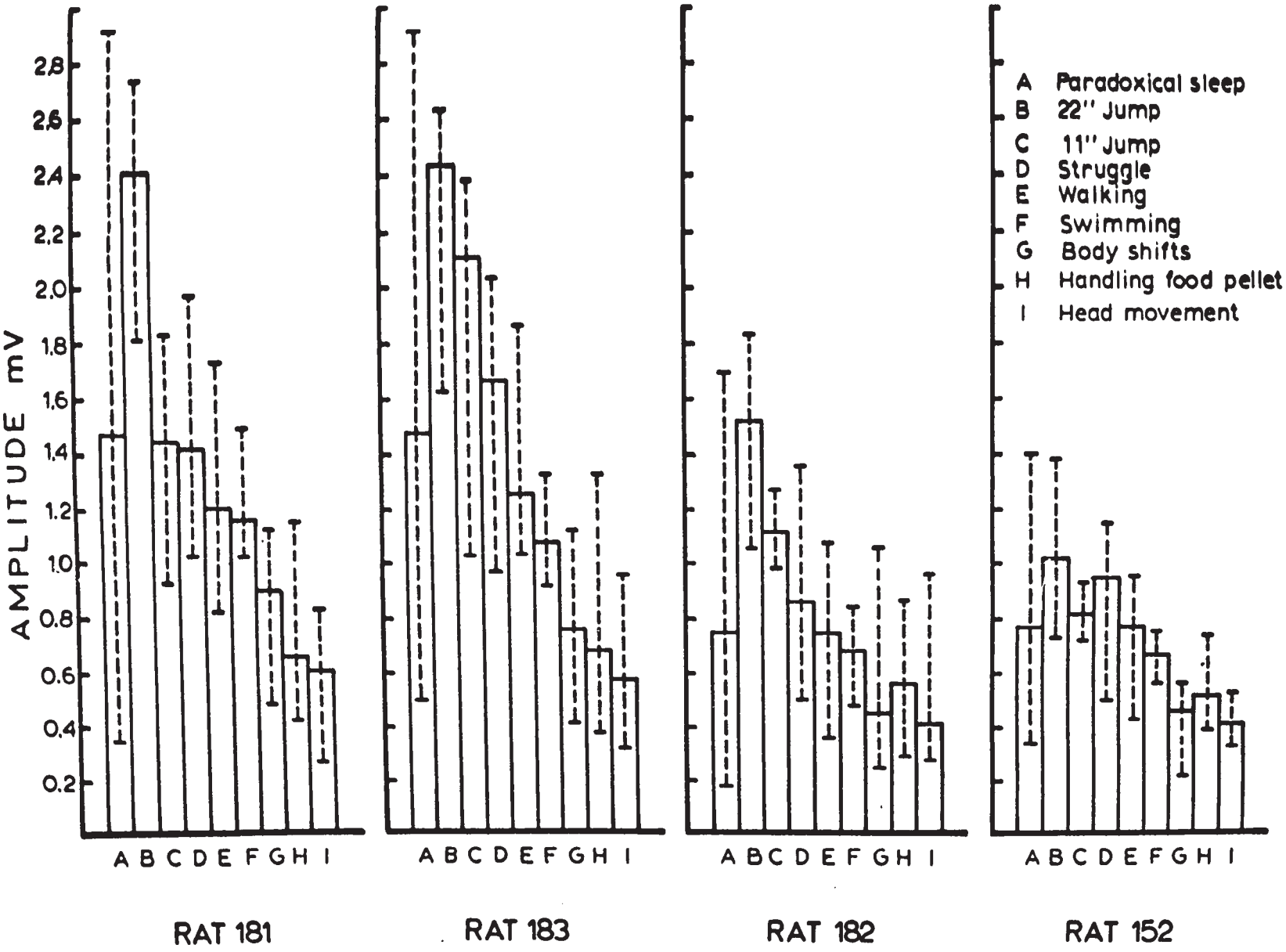
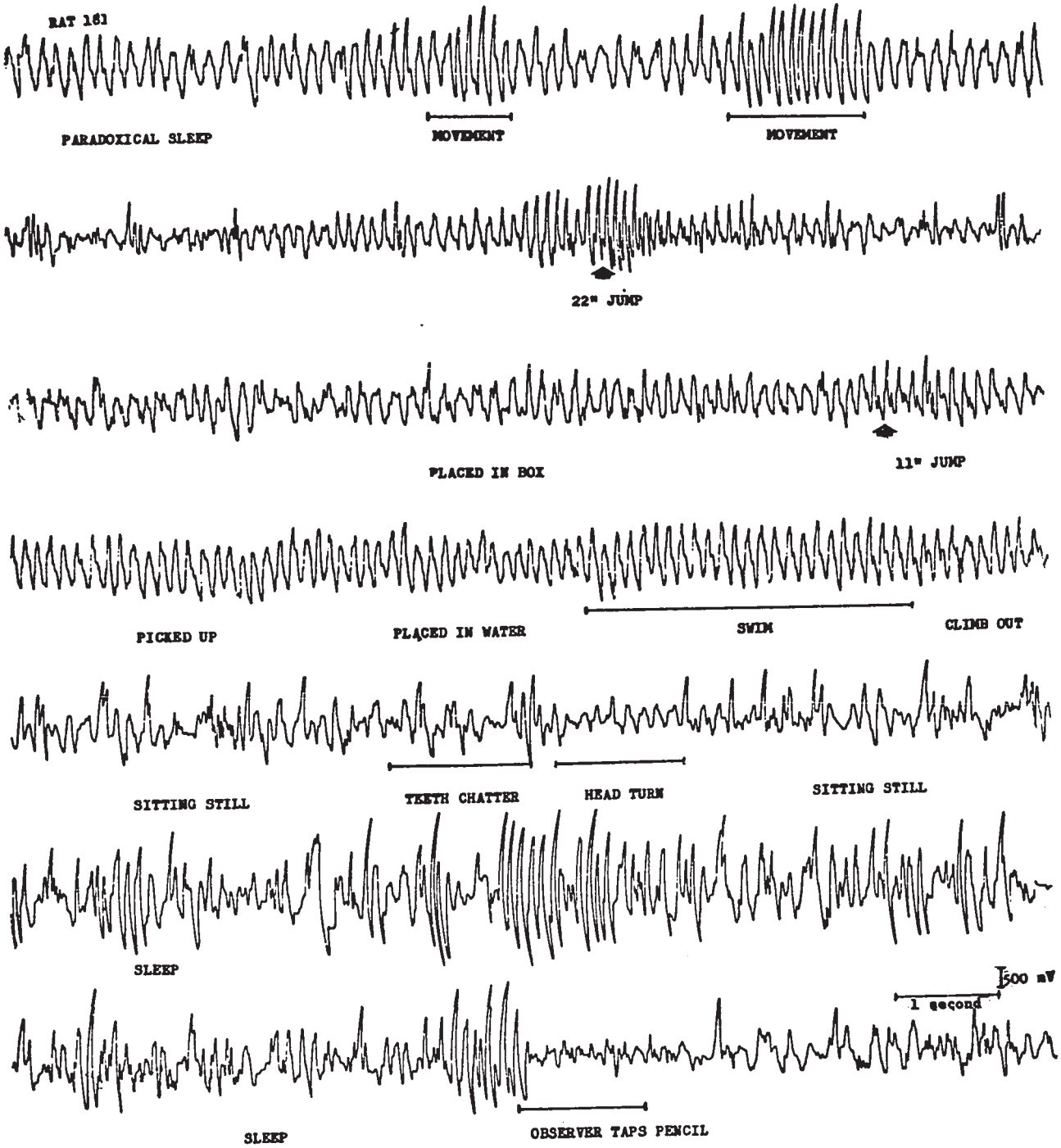

AMPLITUDE mV
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
A B C D E F G H I
A Paradoxical sleep
B 22" Jump
C 11" Jump
D Struggle
E Walking
F Swimming
G Body shifts
H Handling food pellet
I Head movement
RAT 181
RAT 183
RAT 182
RAT 152

Fig. 17. Electrical activity at a single hippocampal site during sleep and various behaviors (rat #181). Note: RSA during paradoxical sleep, jumping, struggling when held in the hand, swimming and head movement; LIA during sitting still while alert and while chattering the teeth; and the irregular slow activity and "spindling" during slow wave sleep; SIA when the rat was awakened but did not move about. Note also: Increased RSA frequency and amplitude associated with twitching during paradoxical sleep and with jumping in avoidance tasks; different frequencies and amplitudes of RSA associated with head movement; swimming, jumping 11 in and jumping 22 in. Cal: 1 sec, 500 μV; half amplitude filters, 0.3 and 75 Hz. Electrode placement: CA 1, hippocampus major.


RAT 161
PARADOXICAL SLEEP
MOVEMENT
MOVEMENT
22" JUMP
PLACED IN BOX
11" JUMP
PICKED UP
PLACED IN WATER
SWIM
CLIMB OUT
SITTING STILL
TEETH CHATTER
HEAD TURN
SITTING STILL
SLEEP
500 uV
1 second
SLEEP
OBSERVER TAPS PENCIL

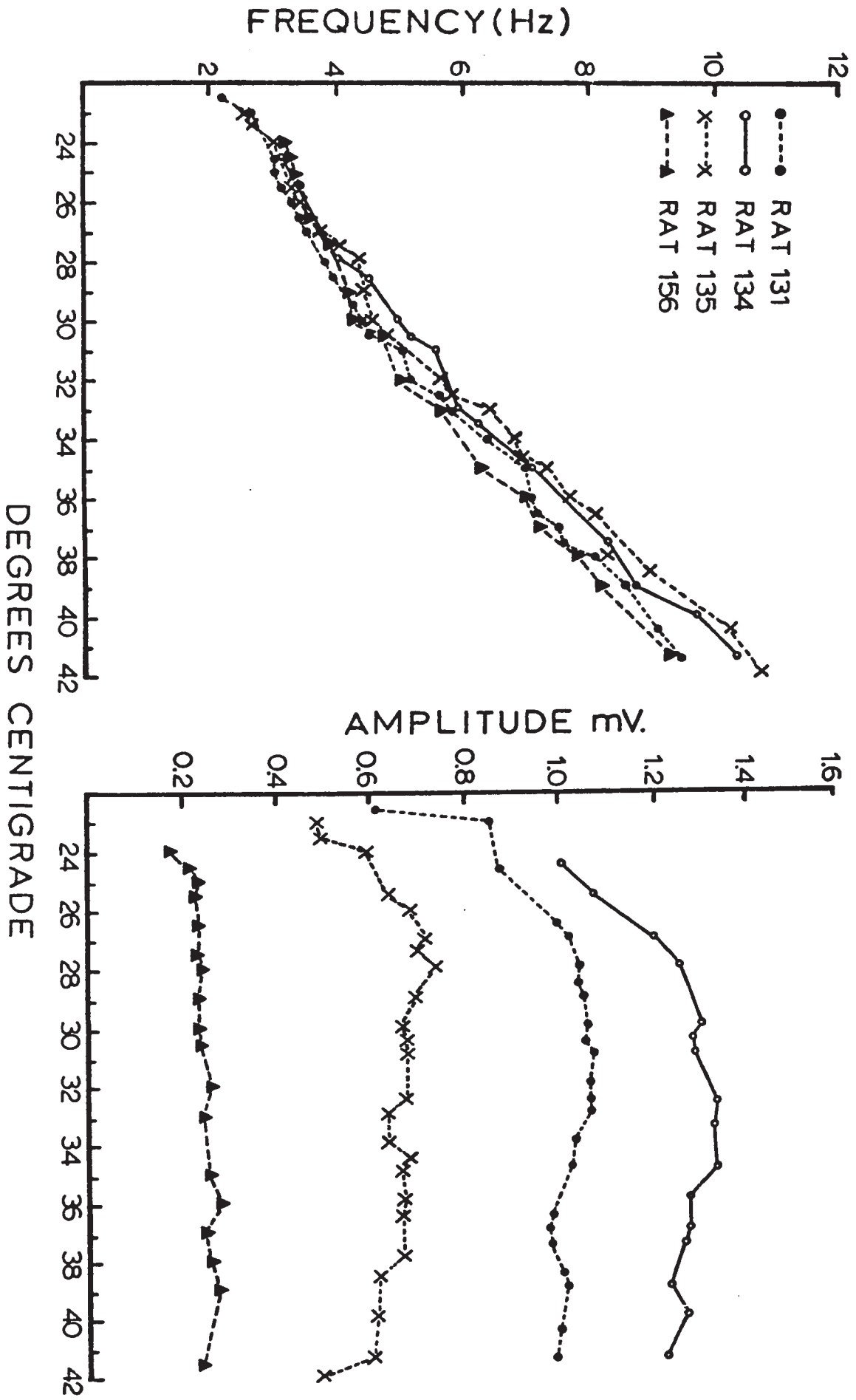
Dissociation of RSA Frequency and Amplitude

Effects of Core Temperature Variation and Fits on Hippocampal EEG

Hippocampal RSA has been found to be directly proportional to core temperature (Weiss, 1963; Whishaw, 1970). In the present study the relation between core temperature and RSA frequency and amplitude was examined. RSA during walking was found to be directly proportional to core temperature, varying from 2-12 Hz over a core temperature range from 23° to 42°C. Amplitude, however, remained unchanged between core temperatures of 26°C to 41°C. Results based on RSA accompanying walking are summarized in Fig. 18. At core temperatures more extreme than 26°C and 41°C RSA amplitude associated with walking declined. No RSA occurred below 23°C and at the same temperature the ability to walk disappears. In addition rats cooled to these temperatures have difficulty maintaining the huddled posture adopted during shivering and violent shivering frequently threw a rat off balance onto its side. Above temperatures of 41°C locomotion resembles crawling although the rats could move quite rapidly. Heating rats to temperatures more extreme than 42°C results in "heatstroke" and the death of the animal within a few moments.

Electrical stimulation of the hippocampus resulted in suppressed activity during stimulation, followed by large amplitude afterdischarges which continued for 10-60 sec after stimulation was termimated. When the afterdischarges ceased the hippocampal record was depressed but gradually recovered in amplitude over 15-30 min. During this recovery period RSA still accompanied movements such as

Fig. 18. Changes in RSA frequency and amplitude in four rats walking at various core temperatures. Data were based on 30-200 waves measured for amplitude and frequency at each point for each rat.


FREQUENCY (Hz)
2
4
6
8
10
12
RAT 131
RAT 134
RAT 135
RAT 156
AMPLITUDE mV.
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
24
26
28
30
32
34
36
38
40
42
DEGREES CENTIGRADE

walking and frequency was the same as that occurring prior to the fit but amplitude was initially markedly depressed.

Hippocampal fits were produced in four rats as they ran in the motor driven wheel at a speed of 60 ft/min. Comparisons of amplitude and frequency of RSA for a 10 sec period during the preictal and postictal stages of the fit indicated that the frequency was unaffected by the fit but that amplitude was reduced following the fit. These results are summarized in Fig. 19.

Fits were also electrically induced in four rats when core temperature was normal and when core temperature was reduced to 26°C or 27°C or increased to 41°C to 42°C. The results are shown in Table 5 and an example from one rat is shown in Fig. 20. The amplitude of RSA associated with walking prior to and following the fit was obtained by measuring frequency and amplitude of 10 sec samples of RSA. RSA frequency during walking was related to temperature in both the normal and postictal states; amplitude was depressed in the postictal state.

The effects of hippocampal fits on behavior were assessed in a number of situations. When fits were induced in animals running in the motor driven wheel, some hesitation in running could be observed during the occurrence of afterdischarges. Following the termination of afterdischarges running seemed to be improved, as the rats ran with fewer pauses. Once RSA had returned to preictal amplitude levels running also returned to normal. Four rats were given fits while they were in the jump avoidance apparatus. These animals had been previously well trained to avoid shock by jumping 11 in. Following the fit the animals engaged in exploration of the box (rearing, sniffing

Fig. 19. Changes in hippocampal RSA frequency and amplitude are shown for four rats prior to (preictal) and following (postictal) an electrically induced hippocampal fit. Fits were induced while the rats were running in the motor driven wheel at 60 ft/min. Data was based on a 10 sec sample of electrical activity preceding and following the fit. It can be seen that frequency is unchanged by the fit while amplitude of RSA is reduced following termination of the fit.

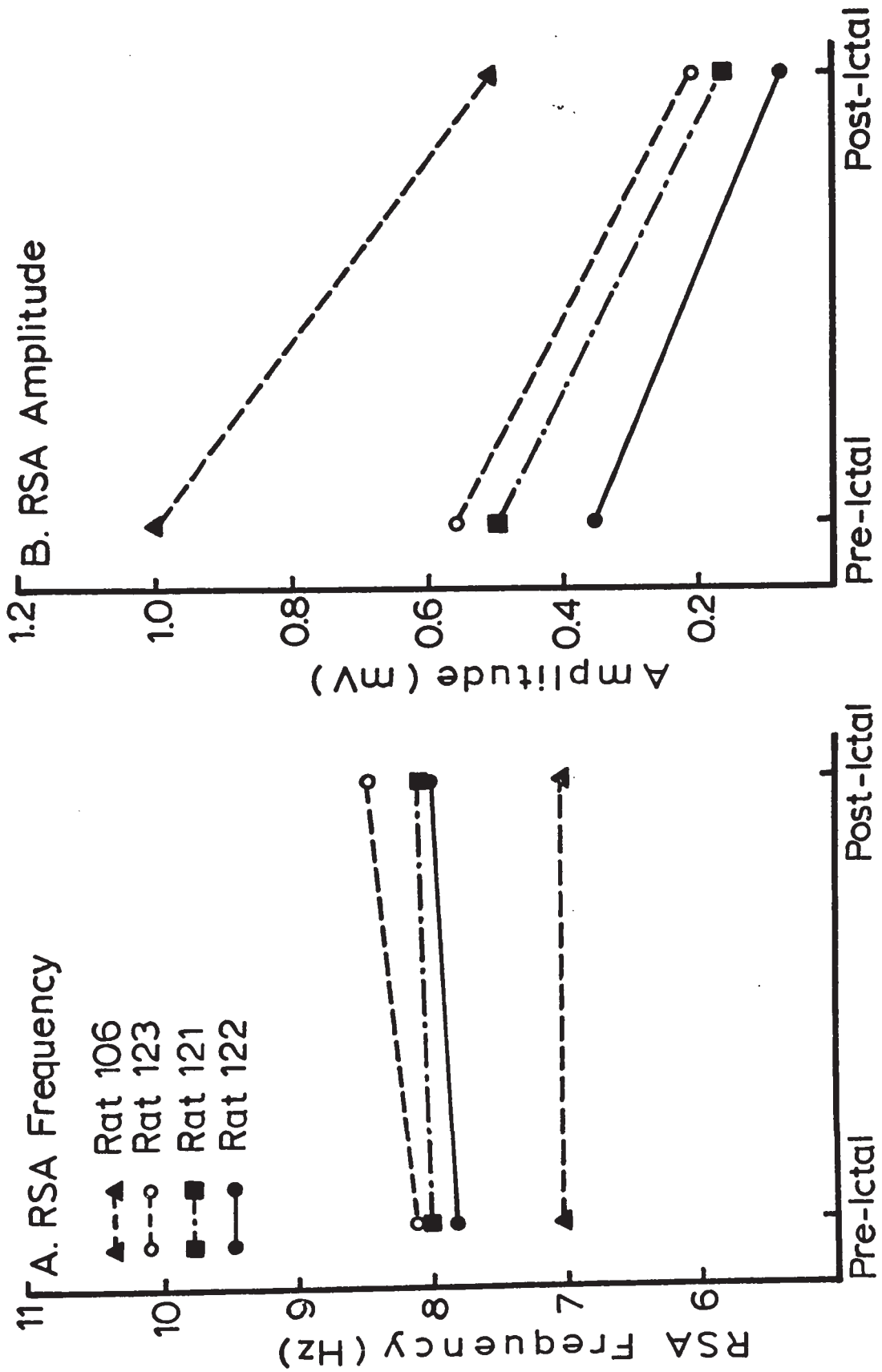
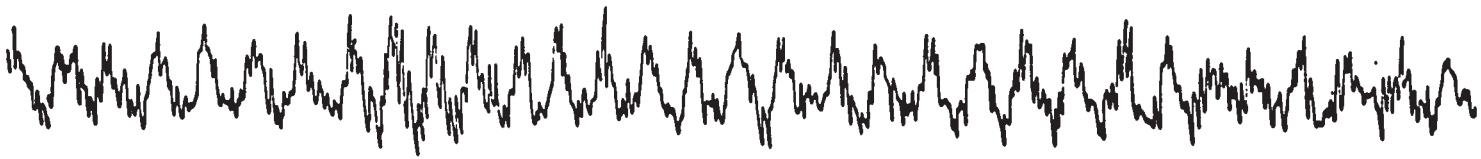

A. RSA Frequency
Rat 106
Rat 123
Rat 121
Rat 122
RSA Frequency (Hz)
11
10
9
8
7
6
Pre-Ictal
Post-Ictal
B. RSA Amplitude
Amplitude (mV)
1.2
1.0
0.8
0.6
0.4
0.2
Pre-Ictal
Post-Ictal

Fig. 20. The effect of changed core temperature and electrically induced hippocampal fits on hippocampal RSA (rat #181). Frequency of RSA varied with core temperature while RSA amplitude remained unchanged. Following a hippocampal electrically induced fit amplitude was reduced in the postictal stage but frequency was related to temperature just as in the normal state. Cal: 1 sec, 500 $\underline{\mu}$V, half amplitude filters 0.3 and 75 Hz. Electrode placement: CA 1 hippocampus major.

CONTINUOUS WALKING - RECTAL TEMPERATURE 37.5°C

CONTINUOUS WALKING - RECTAL TEMPERATURE 42°C



CONTINUOUS WALKING - RECTAL TEMPERATURE 27°C

CONTINUOUS WALKING - POST-ICTAL - RECTAL TEMPERATURE 37.5°C

CONTINUOUS WALKING - POST-ICTAL - RECTAL TEMPERATURE 42°C

CONTINUOUS WALKING - POST-ICTAL - RECTAL TEMPERATURE 27°C

TABLE 5

The Effects of Core Temperature Change and Hippocampal Fits on RSA Accompanying Walking in Rats

		FREQUENCY (MEAN Hz)		AMPLITUDE (RANGE uV)	
RAT #	CORE TEMP.	PREICTAL	POSTICTAL	PREICTAL	POSTICTAL
12	38.0	8.1	8.2	450- 750	50-250
15	38.0	8.0	7.9	350- 525	50-225
20	38.0	8.3	8.1	325- 450	30- 90
181	37.5	7.6	8.0	650-1625	100-375
12	41.0	10.0	10.0	250- 825	100-175
15	41.0	9.6	9.5	275- 550	50-100
20	41.0	9.4	9.2	150- 450	30- 90
181	42.0	9.7	9.4	475-1525	150-300
12	27.0	3.4	3.7	500- 750	25-125
15	27.0	3.4	3.2	350- 550	50- 75
20	27.0	3.3	3.4	215- 425	45- 90
181	27.0	3.2	3.3	500-1500	150-375

[1]Measurements at each point were based on a 10 sec sample of RSA recorded while the rat was walking

and walking) in a compulsive hyperactive manner. They made no avoidances but could be forced to escape by diligent application of foot shock. When they were again replaced in the apparatus they returned to exploratory activities. Normal avoidance did not return with recovery of RSA amplitude but was generally recovered on the following day. Animals given fits at different core temperatures also engaged in hyperexploratory activities although activity level was somewhat reduced at the extremes of core temperature tested. Although shivering was terminated by afterdischarges in cold rats it returned to normal following termination of afterdischarges. Thus, hyperexploratory behavior did not interfere with shivering. Four rats were also food and water deprived for 24 hrs prior to receiving hippocampal fits at normal core temperature. Following the termination of afterdischarges the animals interspersed sessions of eating food pellets and drinking water with periods of exploring, thus the impairment of avoidance did not extend to feeding behavior.

DISCUSSION

Three main types of EEG activity were recorded from single electrode placements in the hippocampus of awake animals. These included rhythmical slow activity (RSA), large amplitude irregular activity (LIA) and small amplitude irregular activity (SIA); patterns similar to those previously described in the rabbit (Stumpf, 1965b), and in the case of RSA and LIA, in the rat (Vanderwolf, 1967, 1969a) and guinea pig (Sainsbury, 1970). These patterns of electrical activity could be distinguished from each other only if an electrode yielded clear RSA. If electrodes yielded mixed RSA and fast activity or mainly fast activity large amplitude RSA could be observed on some occasions during walking, for example, while on other occasions of walking RSA was not recorded. Electrodes yielding clear RSA were often found to be placed with the tips on either side of the hippocampal pyramidal cells, confirming the findings of Green, Maxwell, Schindler and Stumpf (1960), that RSA is generated by these cells. Electrodes yielding mixed or fast activity were displaced above or below this optimal location or placed in the dentate gyrus-CA 4 area, confirming observations of other studies (Adey, 1967; Sainsbury, 1970; Vanderwolf, 1969a). The type of electrical activity recorded from an electrode during a given behavior was stable over long periods of time. For example, observations of hippocampal EEG in rats running daily in a motor driven wheel over a period of months indicated that

the amplitude and frequency of RSA were the same at the beginning and the end of testing. Thus, available evidence seems to indicate that for a given behavior hippocampal activity does not change with time.

Rhythmical Slow Activity (RSA)

RSA Amplitude. At sites in which the recording electrode pair was placed on either side of the pyramidal cell layer RSA was always associated with locomotor behaviors of walking, running, jumping and swimming in the rat. RSA was similarly always associated with walking, digging, running and jumping in the gerbil and with walking in the cat. This relation between movement and RSA was found both when movements occurred spontaneously and when they occurred as a result of formal training procedures in learning tasks. RSA was also always associated with a number of more discrete movements in all species. These included movements of the head alone, postural changes such as occur when an animal changes from grooming one part of the body to grooming another part, and postural adjustments made when the animals were resting. These results confirm and extend findings of other studies in the rat (Vanderwolf and Heron, 1964; Vanderwolf, 1967, 1969a), and guinea pig (Sainsbury, 1970). A similar relation between RSA and movement can be inferred from studies which have used the dog as a subject (Black, Young and Batenchuck, 1970; Dalton and Black, 1968; Storm van Leeuwen, Kamp, Kok and Tielen, 1967; Yoshii, Shimokochi, Miyamoto and Ito, 1966). When the results of the present study and other studies are considered together it can be seen that RSA is related to movement in all species in much the same way; the only

obvious difference in this relation is that RSA frequency in Carnivora is about 2-2.5 Hz slower than in Rodentia. The significance of this latter difference is unknown.

When RSA occurs the waveform varies in both amplitude and frequency but the two parameters are not always well correlated. When rats make slow vertical head raises which often involve both extension of the trunk and limbs, amplitude of RSA can be as large as when the animals run but frequency is lower. Similarly, the frequency of RSA associated with coming to a stop after a rat jumps out of a 22 in box can be as high was 10 Hz while amplitude is only about one half of the amplitude which occurs in the same rat during walking, a movement associated with RSA of a frequency of about 8 Hz.

A more formal investigation of the relation of RSA amplitude to movement in rats indicated that amplitude is related to the size of body movement. During large body movements such as walking, jumping, struggling and swimming the amplitude of RSA is large. In addition, amplitude of RSA associated with jumping out of a 22 in box (the maximum height rats could jump from the restricted grid area) was larger than the amplitude associated with an 11 in jump. The higher jump presumably requires activation of a wider muscular field than the lower jump. Amplitude of RSA during swimming was also somewhat smaller than amplitude of RSA associated with walking; in the former movement the front limbs and trunk are relatively immobile in comparison to the latter movement, again suggesting a relation between extent of muscular activation and RSA amplitude. Amplitude during small movements (discrete lateral head movement, handling food with

the front paws and postural changes during resting) was smaller than the amplitude of RSA which occurred with large movements. In the first two instances the movements did not involve movement of the trunk and hind limbs. In the latter instance although the movement often involved a change in the position of the entire body, the postural change was often accomplished by moving one limb, allowing the body to roll passively into the new position.

Visual inspection of hippocampal EEG records obtained from the gerbil and cat indicated that RSA amplitude was related to movement in much the same way as in the rat; that is, large amplitude RSA accompanied large movements and small amplitude RSA accompanied small movements. In the cat the amplitude of RSA associated with head movements was generally smaller than amplitude of RSA accompanying walking but if the cat raised its head and forequarters while walking (such as occurred as they looked for a way to escape the treadmill) amplitude of RSA was larger than the amplitude associated with walking. The latter difference is probably due to the fact that during walking the trunk of the cat is relatively immobile, while stretching and orienting movements involve greater activation of trunk musculature.

The amplitude of RSA recorded from the hippocampus of the gerbil during movements which occurred in a small observation box was generally smaller than the amplitude of RSA which was associated with running in a motor driven wheel. In the observation box the gerbils moved in short hops (this movement could often be quite rapid) but in the wheel stride was lengthened and the animals galloped. This difference in amplitude between the two movements and situations could

be repeatedly obtained simply by moving the gerbil from one situation to the other. Large amplitude RSA could be observed with some behaviors in the observation box. If a resting animal jumped up and ran across the observation box amplitude of RSA was large with the initiation of running but decreased as the animals continued running and came to a stop. Similarly, if an animal jumped in the air while fighting with another gerbil, amplitude was large during the jump but small or absent altogether in periods between jumps. The amplitude of RSA during head movements in the gerbil was difficult to assess. Head movements were nearly always accompanied by trunk extension, however, if the gerbil sat on its hind limbs and moved only its head, the amplitude of RSA was quite small.

In all of the species examined and in all of the movements described the relation of RSA amplitude to movement was consistent. Movements which were large or involved more extensive muscular activation were associated with large amplitude RSA, while movements which were small were associated with small amplitude RSA. These findings fully confirm the suggestion (Vanderwolf, 1969a) that the amplitude of RSA is related to the extent of muscular activation. The range of amplitudes recorded could be quite extensive. In particularly good electrode placements RSA in the rat could vary sixfold; from waves of 500 μV during a head movement to up to 3 mV during a 22 in jump. This relation between amplitude of RSA and extent of movement emphasizes the importance of this variable of the waveform as well as indicating the necessity of studying the EEG activity with optimally placed electrodes.

RSA Frequency. Examination of the relation of the frequency of RSA to movement showed that both during spontaneous movements and movements performed in formal learning tests frequency increased with movement initiation and decreased as the movement was terminated. In the task in which rats were trained to jump out of a box, they jumped from the grids, caught the ledge with their forepaws and pulled themselves onto the ledge. Prior to the jump, when the rats were sitting still on the grids, the frequency of RSA began to increase steadily for about 2 sec until it reached a peak frequency of 8-10 Hz at which point the jump occurred. When the rat arrived on the ledge the frequency of RSA again decreased. A similar increase in RSA frequency preceded shock avoidance in a one way avoidance task (requiring running behavior) with the peak frequency again occurring at the point the movement was initiated and frequency again decreased as the rats reached the goal area and running was terminated. Initiating running in the motor driven wheel following a pause was similarly associated with an increase in RSA frequency. In this latter case the onset of higher frequency RSA was more abrupt than in the first two tasks and this was perhaps due to the fact that the turning wheel forced the animals to initiate running abruptly. Following the initiation of running the frequency of RSA decreased to remain at a lower and constant level as long as running continued unchanged. Similar changes in RSA frequency were observed to occur with initiation of movements in both rats and gerbils during general movements in an observation box. These results confirm the finding (Vanderwolf, 1969a) that frequency shifts are associated with the initiation of movement and

also show that the shift is a general effect which occurs in association with movement initiation in a number of situations.

The actual frequency associated with movement initiation depended upon the movement. The frequency of RSA preceding, during and following jumping 22 in was significantly higher than the frequency associated with jumping 11 in; mean RSA frequencies in the first situation could reach 12 Hz while in the second, mean frequencies did not exceed 10 Hz. Similarly, when gerbils initiated running at the fastest speed in the motor driven wheel (144 ft/min) frequencies were higher than when they initiated running at the slowest speed (33 ft/min). The differences in frequencies in these situations may be related to the abruptness of movement onset. That is, movements which are initiated very suddenly or rapidly may be associated with higher frequencies than similar movements initiated gradually. This could explain why slow vertical head raises are associated with low RSA frequencies and why bar pressing is similarly associated with lower RSA frequencies than jumping. Small movements of the head alone or paws during food handling are also associated with low frequency RSA (6.6-7.1 Hz; Vanderwolf, 1969a) and again these low frequencies may reflect the gradual onset and termination of these movements.

The frequency of RSA was not related to the speed of a continuous movement. When rats and gerbils were trained to run at different speeds in the motor driven wheel RSA frequency did not increase with speed of running. At all running speeds RSA frequency was the same as that accompanying walking in the wheel at the slowest wheel speed, on an open surface such as a table top, or in a small

observation box. In fact, even though rats trotted at their maximum speed at the third wheel speed (90 ft/min) and gerbils galloped at what appeared to be their maximum running speed at the fourth wheel speed (144 ft/min), the frequency associated with running did not exceed the highest mean frequency (8.3 Hz) recorded by Vanderwolf (1969a) from rats walking in a small observation box. Frequency also did not appear to be related to extent of muscular activation in the way described for amplitude since all movements which continued steadily (swimming, walking, trotting and galloping) were accompanied by similar RSA frequencies (7.5-8.3 Hz). Further, as long as steady movement continued (such as walking in the treadmill for cats and running in the wheel for rats) frequency of RSA remained constant even if testing was continued for as long as 8 hr. This latter effect also indicates that RSA is not subject to habituation as long as movement continues. In summary, frequency of RSA changes only when movement changes, with greater increases occurring with more abruptly initiated movements and declining frequencies associated with the termination of movement. Once steady movement occurs, frequency remains steady at what may be considered a base level (7.5-8.3 Hz), irrespective of movement speed, as long as movement continues.

In a number of situations RSA was recorded from both cats and rats during periods when no visible movements occurred. Prior to jumping out of either a 22 in or 11 in box RSA was recorded from rats although careful observation indicated they did not move. In these situations frequency of RSA increased steadily for 1-2 sec until the rats jumped, suggesting that central mechanisms responsible for

movement initiation were active but that activation had not reached a level necessary to trigger the appropriate movement. RSA was also recorded from rats during an immobility or freezing response following procedures during which a tone was paired with inescapable foot shock. Mean frequencies in this case were extremely low; less than frequencies associated with any observed movement. Similarly, RSA was observed during fixed staring in cats and Brown (1968) has reported that RSA frequency at this time is significantly lower than RSA frequencies which occurred at other times when the cats were, presumably, moving. In both these cases the low frequencies may indicate that movement is programmed centrally in a way similar to that occurring during an early phase of movement initiation but that the movements are never triggered. The regularization of respiration which occurred during the conditioned immobility response in rats may also be indicative of a general readiness for movement.

The frequency of RSA during various phases of movement may be related to the activity in complex neural circuits responsible for the initiation and maintenance of movement. Electrical stimulation of the brainstem reticular formation has been found to produce hippocampal RSA (Green and Arduini, 1954) and later physiological studies have indicated that the ascending pathways run through the medial hypothalamus and medial septum (Corrazza and Parmeggiani, 1963; Petsche, Stumpf and Gogolak, 1962; Torii, 1961; Yokota and Fujimori, 1964) or through the medial thalamus and medial septum (Eidelberg, White and Brazier, 1959; Kawamura, Nakamura and Tokizane, 1961) to the hippocampus. In acute preparations, a direct relation has been found

between the intensity of electrical stimulation applied to brainstem structures and the frequency of RSA at stimulation onset (Sailer and Stumpf, 1957; Weiss, 1964), while in studies with chronic preparations stimulation of subcortical sites has been found to induce running as well as hippocampal RSA (Bland and Vanderwolf, 1970a). Such results seem to support the idea that movements are initiated by subcortical structures (Penfield, 1954), the activation of which also produces hippocampal RSA.

The findings of the present study, that frequency of RSA does not increase with running speed, suggest that there is no simple relation between the activity in brainstem structures which activate movement and the frequency of hippocampal RSA since it would be expected that faster running would be a result of greater brainstem activity. Other physiological evidence supports this interpreation. Although a direct relation between RSA frequency and intensity of brainstem stimulation has been found at the onset of stimulation, with continued stimulation at all voltages RSA frequency declines to a lower level (Sailer and Stumpf, 1957). A similar effect is obtained when freely moving animals are placed in a wheel and electrical stimulation is applied to the posterior hypothalamus. Stimulation voltage, RSA frequency and running speed are directly related at stimulation onset but with continued stimulation at any voltage, running speed is maintained while RSA frequency declines to a base level of about 8 Hz (Bland and Vanderwolf, 1970a). Thus, once movement is initiated, galloping in response to high voltage stimulation and walking in response to low voltage stimulation are both accompanied by similar

RSA frequencies. These results support the present interpretation that the hippocampus responds with a constant RSA frequency to indicate steady movement irrespective of response speed or movement pattern, yet reflects changes in movement with frequency shifts.

It has been demonstrated that the integrity of the midbrain is necessary for spontaneous walking to occur in rats (Woods, 1964). It would also be expected that increased excitation in midbrain structures is responsible for locomotion and that a relation would exist between midbrain excitation and naturally occurring or stimulation induced running at different speeds. The finding that hippocampal RSA reflects changes in movement but not movement speed seems to indicate that a postulated relation between midbrain activity and running speed does not occur between midbrain activity and RSA frequency. It would seem, therefore, that brainstem activation of hippocampal RSA is modified, possibly at the level of the septal pacing cells or hippocampus itself so that the hippocampus maintains a sensitivity to changes in subcortical activity and movement. Implicit in this conclusion is the idea that modification of neural activity in the multisynaptic pathway between the brainstem and hippocampus facilitates the role of the hippocampus in some sort of control or guidance of certain kinds of movements.

The Independence of RSA Amplitude and Frequency

In the preceding discussion it has been suggested that amplitude and frequency of RSA represent different mechanisms. This independence of the two parameters was suggested by their different

relation to movement as well as observations of a lack of correlation during certain movements. This conclusion was also supported by the finding that there is no correlation between amplitude and frequency of individual waves (Vanderwolf, 1969a). Brainstem mechanisms probably control both amplitude and frequency of RSA but it has not yet been demonstrated that different systems have different effects on the two parameters. Kawamura and Domino (1968) have demonstrated, however, that stimulation in different sites can have different effects on hippocampal activity. Stimulation of the medial thalamus produces both lower frequency and lower amplitude RSA than is produced by stimulation of the hypothalamus.

Amplitude and frequency of RSA can be manipulated independently by other procedures. Between core temperatures of 26-41^{o}C the frequency of RSA in rats is directly proportional to core temperature with lower core temperatures producing lower, and higher core temperatures producing higher RSA frequencies. Within this range of core temperatures amplitude remains constant but declines at more extreme temperatures. Present evidence indicates that these changes are due to the direct effects of core temperature changes and not to variables, such as fatigue, resulting from cooling procedures (Whishaw, 1970). In this study, rats with unclipped fur were required to swim for the same length of time as clipped rats and the animals in the two groups, did not show the same degree of change in core temperature or EEG. In addition, when rats were rewarmed while swimming in warm water the EEG returned to normal as body temperature rose to normal. Alternately, in the postictal period of an electrically induced hippocampal

electrographic seizure, amplitude of RSA is depressed while frequency of RSA in unchanged from the preictal phase. This latter effect was demonstrated in rats running in the motor driven wheel at normal core temperatures as well as in rats walking at various core temperatures. These results support the idea that amplitude and frequency are independent parameters of RSA by demonstrating that they can be manipulated independently.

The neurological mechanisms underlying normal variations in frequency and amplitude of RSA are completely unknown. A simple speculation is that amplitude is an index of the number of cells participating in the rhythm, while frequency is an index of the rapidity with which excitation-inhibition cycles such as those described by Euler and Green (1960) and Spencer and Kandel (1962) succeed each other. The effects of core temperature on RSA frequency is consistent with the idea that frequency is related to the rapidity of changes in the excitability of cells. Cooling has been reported to slow the firing rate of neurons (Chatfield, Battista, Layman and Garcia, 1948; Gasser, 1931) and a similar effect may account for slowing of RSA during hypothermia. On the other hand, temperature changes may not affect the number of cells which are active, thus, accounting for the lack of change in amplitude. Electrographic seizures have been reported to produce prolonged depolarization of pyramidal cells (Spencer and Kandel, 1962). Therefore, reduced amplitudes of RSA in the postictal phase of an electrographic seizure may be due to a reduction in the number of active cells, while unchanged frequency may indicate that the cells which remain active still maintain normal

excitability cycles.

Large Amplitude Irregular Activity (LIA)

Large amplitude irregular activity was recorded from all species examined during a number of behaviors. In general, the amplitude of this activity was similar to the amplitude of RSA accompanying walking with the exception both amplitude and frequency of waves were completely irregular from wave to wave. LIA was recorded from the rat, cat and gerbil during alert immobility and during periods when the animals were resting but not sleeping. This confirms the findings of Vanderwolf (1969a) in rats and Sainsbury (1970) in the guinea pig. Since LIA has also been reported in rats hanging immobile from a ledge (Vanderwolf, 1969a) the activity appears to be a correlate not only of relaxed immobility but also immobility associated with isometric muscle tension. During feeding in all three species, LIA was recorded during chewing food and during water lapping in the rodents and milk lapping in the cat. The suggestion has been raised in a number of papers (Gray and Ball, 1970; Komisaruk, 1970; Paxinos and Bindra, 1970) that RSA does in fact occur during water lapping or food chewing, but that the amplitude is too small to be observed or the activity is "residual." Close examination of LIA during these movements indicated that amplitude was as large as the amplitude of RSA accompanying walking and that there was no relation between the EEG pattern and the rhythmical movements. An explanation for the contradictory reports may be that in these experiments the animals were also making other movements of the head or body when RSA was

observed during chewing or water lapping, in which case RSA would occur.

During grooming, both rats and gerbils engage first in one grooming act and then make a postural adjustment and engage in another grooming act. For example, an animal may first rub its nose with both front paws, then rub down its face, following which the animal makes a postural adjustment and then engages in an act of grooming some other body part. The entire sequence is highly organized and given that one grooming sequence occurs the following sequence is, to some extent, predictable (Hopkins, 1970). Hippocampal recordings taken from animals during grooming indicated that LIA occurred during face washing and both during fur licking and fur chewing as well as genital cleaning and tail cleaning. However, during the postural adjustments between various acts of grooming RSA was recorded. These results confirm previous findings in the rat (Vanderwolf, 1969a) and guinea pig (Sainsbury, 1970). LIA was also recorded during conspecific grooming in the gerbil and during foot stomping in the gerbil (also reported by Kramis and Routtenberg, 1969) as well as during scratching in the rat and gerbil. The pattern of LIA recorded during various behaviors including drinking, chewing, grooming and scratching as well as immobility appeared similar and it was not possible to distinguish one behavior from another by visual examination of the records.

A general explanation for the relation of LIA to behavior is that it signifies that the hippocampus is not active or does not partake in the organization of the behavior. This interpretation is

consistent with the results indicating that LIA occurs during immobility. The finding that LIA also occurs during a variety of stereotyped, rhythmical movements which are generally referred to as being automatic (Pickenhain and Klingberg, 1967; Routtenberg, 1968; Vanderwolf, 1969a) may indicate that the neural circuits which control these movements are organized in the brainstem and do not involve the forebrain.

The idea that LIA represents an idling state of the hippocampus or a state of inactivity is supported by other evidence. Electrographic seizures produced by stimulation of the hippocampus of the cat have been found not to interrupt the lapping movements made by a cat drinking milk (Akert, 1961), however, they do prevent the occurrence of avoidance response in the cat (MacLean, Flanigan, Flynn, Kim and Stevens, 1955; Flynn and Wasman, 1960). In the first case the electrographic seizure would interrupt LIA but not the behavior while in the second case both RSA and behavior would be interrupted. Dentate-CA 4 stimulation has been found to suppress the electrical activity of the hippocampus as well as arrest avoidance behavior in the rat. Similar stimulation does not interrupt water lapping (Bland and Vanderwolf, 1970b). Shivering in the rat has also been found to be associated with LIA and if an animal is cooled below 23^{o}C, RSA and movements such as walking no longer occur, while shivering can occur at core temperatures as low as 16^{o}C (Whishaw, 1970). In addition, dentate-CA 4 electrical stimulation can arrest the movement of a slightly cooled rat pulling itself up on a ledge by its forepaws while shivering continues uninterrupted throughout stimulation (Whishaw and

Bland, unpublished data). Finally, lesions to the hippocampus of both the rat and gerbil affect behavior associated with RSA (usually resulting in an increase of "release" in the frequency of occurrence of the behaviors) but do not have a similar effect on behaviors associated with LIA (Glickman, Higgins and Isaacson, 1970; Kim, Choi, Kim, Chang, Park, and Kang, 1970). In all of the instances cited, manipulations which disrupt hippocampal activity disrupt movements associated with RSA in some way, while a similar effect does not extend to movements associated with LIA. Therefore, the hippocampus probably does not play a role in the control of movements associated with LIA.

One of the original suggestions of Green and Arduini (1954) was that RSA was a sign of hippocampal arousal just as cortical desynchronization was a sign of cortical arousal. The findings of the present study, that RSA changes with changes in movement, support this interpretation. Electrophysiological studies which indicate that RSA is associated with excitatory-inhibitory cycles in pyramidal neurones (Andersen, Eccles and Lyning, 1963; Euler and Green, 1960; Fujita and Sato, 1964; Spencer and Kandel, 1962) as well as anatomical evidence indicating that the main efferent path from the hippocampus is via the axons of pyramidal cells (Crosby, Humphrey and Lauer, 1962; Blackstad, Brink, Hem and Jeune, 1970) is also consistent with the idea that RSA indicates that the hippocampus is active. This evidence is difficult to reconcile with the idea that RSA represents hippocampal inactivity (Grastyán, Lissák, Madarász and Donhoffer, 1959). On the other hand, both behavioral-EEG correlations and other physiological

evidence outlined above is consistent with the suggestion that LIA indicates an idling or inactive hippocampal state.

Small Amplitude Irregular Activity (SIA)

Small amplitude irregular activity (SIA) is a depressed hippocampal waveform with an irregular amplitude and frequency. For the purposes of definition any suppressed activity with an irregular pattern and an amplitude as small or smaller than the amplitude of RSA associated with a discrete lateral head movement was defined as SIA. SIA was studied in both rats and gerbils. SIA was recorded from the hippocampus of rats, at points which also yielded clear RSA, on occasions when they jumped in the motor driven wheel and then rode back in the wheel; the pattern occurred when the rats were immobile on the ride back. SIA was also recorded as rats arrested the forward motion of their body to seize a food pellet following a bar press and when they stopped running in the motor driven wheel and remained immobile until the movement of the wheel forced them to run again. When sleeping rats (both the neocortex and hippocampus showed high voltage slow activity) were presented with a tone or were aroused by the noise of a tapping pencil, the neocortex desynchronized and at the same time SIA appeared in the hippocampus, provided the animals did not move. RSA occurred if the rats moved their heads or limbs. In the mongolian gerbil SIA appeared in the hippocampal record when a gerbil introduced into the observation box adopted an immobile posture upon meeting another gerbil which was already present. SIA was also often recorded from gerbils during concentrated sniffing when other body movements did

not occur and during mating with pelvic thrusting.

Since SIA was associated with immobility or the arrest of movement associated with RSA the electrical pattern may be a hippocampal sign of movement inhibition. SIA was not recorded when rats or gerbils were sitting still or when rats were conditioned to remain immobile with inescapable foot shock, therefore the pattern could not be a simple correlate of immobility. Since SIA was also recorded during pelvic thrusting and concentrated sniffing in gerbils (automatic activities) possible inhibitory effects could not extend to these kinds of movements. In the latter cases both movements require that the animal adopt a special posture (in the case of concentrated sniffing the gerbil frequently arches its back until it is bent nearly double and then bobs its head to sniff) which may require inhibition of some sort to maintain. This interpretation is supported by the observation that during genital sniffing the animals do not adopt a posture and LIA accompanies sniffing. The finding that SIA occurs when resting rats are aroused but do not move confirms previous observations in the rat (Pickenhain and Klingberg, 1967; Vanderwolf, 1969a), the cat (Brown, 1968; Grastyán, Lissák, Madarász and Donhoffer, 1959) and rabbit (Green and Arduini, 1954). The occurrence of SIA at this time may indicate that the arousing stimulus would cause the animal to get up and run were it not for inhibition processes which allow the animal to remain still long enough to assess the significance of the disturbance. This latter interpretation is supported by the finding that septal stimulation produces hippocampal SIA as well as arrest of movement (Ito, 1966), while septal lesions produce a

hyperreactivity in the rat which can in extreme cases cause a disturbed rat to leap up and bound away (Brady and Nauta, 1953).

Other evidence also supports the interpretation that SIA is a correlate of some process of activity inhibition. Electrical stimulation of the anterior lateral hypothalamic area suppresses hippocampal RSA as well as suppressing spinal reflexes (Yokota and Fujimori, 1964). Electrical stimulation of the dentate gyrus-CA 4 area suppresses hippocampal RSA and jumping but does not suppress movement of lapping water (Bland and Vanderwolf, 1970a) or shivering (Whishaw and Bland, unpublished data). These results indicate that electrically induced suppression of RSA is similar in pattern to naturally occurring SIA and that the behavioral correlate, inhibition of movements of the type normally associated with RSA, in analogous. It can be concluded from these results that the electrical activity of the hippocampus reflects not only the activity of mechanisms associated with movement but also mechanisms responsible for the inhibition of movement.

Hippocampal Electrical Activity During Sleep

Hippocampal electrical activity in the rat during slow wave sleep is similar to that described in the rabbit (Green and Arduini, 1954) during slow wave sleep. Amplitude of the EEG is larger than LIA observed in the waking animal and slow waves are periodically interspersed with spindles with a frequency of about 12 Hz and an amplitude larger than the largest RSA waves observed in the animals.

During paradoxical sleep long trains of RSA occur but the only movements observed are slight twitches of the animal's extremities

(paws, ears and vibrissae) and occasional twitches of the back. Usually all movements occur together and periods of movement can last from less than a second to about ten seconds. Between periods of twitching no movements are observed. Frequency and amplitude measurements of RSA from records obtained from rats indicated significantly higher frequencies and amplitudes of RSA occurred during periods of twitching than during periods of non-twitching. On the basis of the relation of frequency and amplitude to movement in awake rats it would be expected that the sleeping rats should be running and jumping rather than sleeping. Pompeiano (1967) has reported that high levels of activity occur in cerebrospinal (motor) pathways during paradoxical sleep. Movements are apparently prevented by a tonic postsynaptic inhibition of spinal motor neurons throughout paradoxical sleep and by a phasic presynaptic inhibition during movement periods of paradoxical sleep. Paradoxically, descending excitation is great enough to break through both types of inhibition and result in twitches. Selective suppression of descending inhibition with lesions to the brainstem result in cats which awaken and display "hallucinatory" behavior rather than entering the paradoxical sleep state (Jouvet, 1967).

The correlation of large amplitude high frequency RSA with twitches of the extremities, together with the findings of Pompeiano (1967) indicate that central mechanisms involved with movement are extremely active during paradoxical sleep. Yoshii, Shimokochi, Miyamoto and Ito (1966) have also recorded large amplitude high frequency RSA during movement periods in the dog while Okuma and Haruo (1966) report similar changes in the cat and Parmeggiani and Zanocco

(1963) report such changes following movement in the cat. The interpretation by Yoshii et al. (1966) that amplitude changes may be related to hormonal changes is doubtful in view of the relation of amplitude to movement size in the awake moving animal. It is also difficult to assess the contradictory reports by Okuma and Haruo (1966) and Parmeggiani and Zanocco (1963) on the temporal relation of RSA change to movement in the cat since these reports were based on passing observation rather than systematic examination. It is likely that future studies will show that RSA changes during paradoxical sleep in other species are similar to the changes which occur in the rat. Further support for the present findings is provided by observations and EEG recordings taken from sleeping cats following sleep deprivation. Kiyono, Kawamoto, Sakakura and Iwamura (1965) report an overall increase in RSA frequency during "makeup" paradoxical sleep and Jouvet (1965) has described twitching as increased so markedly at this time that the animals appear to be having an "epileptic seizure." Although the significance of central activation of motor systems during paradoxical sleep is not known it has been suggested that dreaming in humans occurs during paradoxical sleep (see Kleitman, 1963). Possibly, the animals in the present study were dreaming of running and jumping. At any rate, the occurrence of RSA and the changes in the pattern of RSA during paradoxical sleep with movements does appear to indicate that central motor systems are active during paradoxical sleep in a way much the same as that observed in the awake animal.

The Relation of Hippocampal EEG to Voluntary Movement

A general interpretation of the relation of hippocampal EEG to behavior suggests that RSA is a correlate of voluntary movement while LIA is a correlate of more automatic movements and immobility (Vanderwolf, 1969a, 1969b). The results of the present research are consistent with this interpretation in all species. In addition, a third waveform SIA has been examined in the rat and gerbil and found to be related to the inhibition of voluntary but not automatic movements.

One of the original ideas of Jackson (see Taylor, 1958) was that parts of the body were represented at a number of levels in the nervous system in such a way that the anatomically highest centers represented the most complex and coordinated movements while more reflexive or automatic movements were organized in anatomically lower nervous system centers. Vanderwolf (1969a, 1969b) has suggested that part of the difference between automatic and voluntary movements is that automatic movements may be dependent on the occurrence of a particular motive state, while voluntary movements may be controlled in the presence of a number of motive states. In order for movements to be selectively controlled under various motive states, such as hunger, thirst or fear, complex circuitry in the forebrain (hippocampus and neocortex) may be necessary for switching and guiding movement. Since activities such as walking can readily be controlled by a number of motive states and are always associated with the occurrence of RSA in the hippocampus, RSA has been suggested as a correlate of voluntary movement. On the other hand, activities

termed automatic such as grooming are relatively stereotyped, more difficult to condition and are associated with LIA.

A number of other lines of evidence support a classification of movements as either voluntary or automatic. Mesencephalic rats will groom if a spot of water is placed on their fur and normally keep the fur clean. They will also chew food and lap water if it is presented to their mouths but though they can walk, they will not search out food or water once it is moved a few inches away (Woods, 1964). Thus, grooming still serves its biological function of keeping the animals clean, while walking no longer serves its function of getting the animals to food and water. A similar organization of movements has been demonstrated in cats (Bard and Macht, 1958; Wang and Akert, 1962, Woods, 1964) but in these animals the striatum appears necessary for grooming. The difference between rats and cats indicates that behaviors are organized at different levels in the two species, nevertheless, these examples illustrate that following extensive lesions some movements retain a functional utility while other movements, though still present, no longer serve a purpose. In all cases the movements or behaviors which are most severely impaired are those associated with RSA.

Electrographic seizures which result in functional ablation of the limbic system (Delgado and Sevillano, 1961; MacLean, Flanigan, Flynn, Kim and Stevens, 1955-56) have a greater disruptive effect on voluntary behaviors than involuntary behaviors. In the present study it was found following a hippocampal electrographic seizure (fit) well trained rats no longer performed a shock avoidance response. This

deficit did not appear due to a disruption of motor coordination since the animals engaged in exploratory behavior in the apparatus and in an observation box, while in a running wheel, they actually showed improved running. The animals would also make an escape response if foot shock was administered. The effect could not have been due simply to interference of hyperexploratory behavior since food and water deprived animals still ate and drank following a fit and cold rats still shivered. The failure of the animals to avoid seems to indicate that fits selectively disrupt a more voluntary learned response. The increase in exploratory behavior and improved running is probably due to a "release" of subcortical structures from cortical control. Failure to find a similar disruption in eating and drinking and shivering suggests that these responses are under lower level control only. In fact, following electrically induced hippocampal fits MacLean (1957) has reported eating and drinking in non-food deprived animals and suggested that this eating and drinking is a result of a "release" of the hypothalamus from hippocampal control.

The electrophysiological evidence reported here and elsewhere (Sainsbury, 1970; Vanderwolf, 1969a) only divides movements into rather general categories of voluntary and automatic. Undoubtedly gradients exist as suggested by Jackson, however, other lines of evidence may be necessary for a more detailed classification. Grooming in the cat, for example, could be called more voluntary than scratching since the striatum is necessary for grooming but spinal cats can still show a scratch reflex. Shivering in the rat is probably also more automatic than grooming since shivering occurs in a rat cooled until

it is unable to walk or groom (Whishaw, 1970). Finally, a learned response such as a shock avoidance response is probably more voluntary than behaviors such as exploration, even though the same motor patterns may be used in both, since the former is disrupted and the latter "released" by an electrically induced electrographic seizure. Many such examples could be described, but for present purposes it is probably sufficient to conclude that movements associated with RSA are more voluntary than movements associated with LIA.

The role of the hippocampus in behavior is not known but present evidence indicates that it plays a part both in the initiation and arrest of voluntary movement. A possible function may be that it acts as a switch in complex circuits responsible for selecting responses appropriate to various motive and different environmental conditions. The significance of RSA may be not that it is just a correlate of voluntary movement, but that it indicates that these movements can readily be brought under control of a number of motive states or sensory cues.

Other Interpretations of Hippocampal EEG

Previous interpretations of hippocampal EEG have related changes in hippocampal activity to learning. Grastyán, Lissák, Madarász and Donhoffer (1959) have suggested that the disappearance of RSA during practice on either avoidance or approach responses indicated that desynchronization of the hippocampal EEG was a sign of hippocampal activation while RSA was a sign of hippocampal inactivity. The results of the present research, however, indicate that

RSA will disappear during practice on a learning task only if the response involves reduction in movement and electrodes are displaced from the pyramidal cells or located in the dentate gyrus-CA 4 area. This was demonstrated specifically by training rats to either run in a motor driven wheel or bar press for food. In both situations the animals were given extensive training but wheel running always required a large body movement, while during bar pressing movement size could be reduced with practice as the rats learned to stop walking about and sit near the bar pressing with one paw. RSA was found to be always present in all recording electrodes in the hippocampus during wheel running, while during bar pressing RSA disappeared from mixed placements but persisted at reduced amplitude at clear RSA placements. The present conclusion, that RSA does not disappear with training if the electrode is optimally placed, is further supported by the finding that true hippocampal "desynchronization" or SIA is associated with the inhibition of voluntary movement.

On the basis of changes in the frequency of RSA, Elazar and Adey (1967) have related hippocampal EEG changes to attentional processes. Higher frequencies of RSA were recorded from cats during correct responding on a two choice discrimination task late in training than during either early training or incorrect responses late in training. The results of the present study demonstrate quite clearly that changes in the frequency of RSA are related tc changes in movement. The lower RSA frequency recorded by Elazar and Adey (1967) during incorrect trials may have been due to the occurrence of more irrelevant movements (pauses or head movements). Longer

response latencies are more likely to be obtained at these times and head movements and pauses are more likely to occur (Tolman, 1948). The association of lower RSA frequencies with head movements and pauses and the higher frequencies of RSA with a direct approach probably account for the differences reported and such an interpretation is more parsimonious than an attentional hypothesis. Other attempts to correlate higher frequencies of RSA obtained from food deprived rats with attentional processes (Ford, Bremner and Richie, 1970) could also more easily be attributed to increases in movements which would be expected from deprived animals (Siegal and Steinberg, 1949).

More recently Komisaruk (1970) has suggested that RSA in rats is a correlate of rhythmical activities such as heart rate, vibrissae movements, sniffing and possibly chewing food and lapping water. Examination of heart rate indicated that there was frequently no phase relation. Similarly, there was not always a phase relation between movements of the vibrissae and RSA. During swimming, rats did not move their vibrissae and clear RSA was recorded. Cats also did not move their vibrissae during walking and RSA was always recorded at this time. During concentrated sniffing in the mongolian gerbil SIA and not RSA was recorded and Vanderwolf (1969a) has reported that LIA occurs during sniffing in the rat if the act is unaccompanied by head movements. Finally, LIA was recorded from all three species during chewing food and lapping water or milk and there was no indication that RSA was either too small in amplitude to be to be observed or that it was "residual." These results give little support to the interpretation that RSA either generates or is a result of these rhythmical activities.

CONCLUSIONS

The results of the present research indicate that the hippocampal EEG in the three species studied reflects movements made by the animals. RSA occurs during voluntary movement (as defined by Vanderwolf, 1969a, 1969b); amplitude reflecting the size of body movement and frequency the abruptness of movement onset. LIA occurred during automatic movements and immobility which suggests that the pattern indicates hippocampal inactivity. SIA occurred during immobility following sudden termination of voluntary movements, when sleeping animals are aroused but do not move and during certain automatic movements (in the gerbil). The relation of SIA to movement seemed to indicate the pattern represented the active inhibition of voluntary movement.

Stumpf (1965b) has described two mechanisms which act on the hippocampus, one generating RSA and the other suppressing RSA. The present findings indicate that mechanisms activating RSA also activate voluntary movements while mechanisms which suppress RSA arrest voluntary movement. The reflection of this dual control of movement in the hippocampal EEG suggests that the hippocampus plays a part in the control of voluntary movement. A possible function of the hippocampus in the higher control of movement is to link the appropriate movement to various motive states and environmental conditions.

No support was found for suggestings that hippocampal EEG changes with learning independent of changes in movement. If recording electrodes were placed across the pyramidal cell layer of the hippocampus RSA did not disappear with practice on learning tasks. Frequency shifts occurred with the initiation and termination of movement and could not be interpreted as an indication of attentional processes. Finally, no support was found for suggestions that RSA either generates or reflects rhythmical activities such as vibrissae movements, heart rate, sniffing, chewing or lapping water.

REFERENCES

Adey, W. R. Hippocampal states and functional relations with cortico-subcortical systems in attention and learning. In W. R. Adey and T. Tokizane (Eds.) Progress in Brain Research. Structure and Function of the Limbic System. Amsterdam: Elsevier, 1967, 27, 228-245.

Adey, W. R. & Dunlop, C. W. The action of certain cyclohexamines on hippocampal system during approach performance in the cat. Journal of Pharmacological and Experimental Thereputics, 1960, 130, 418-426.

Adey, W. R., Dunlop, C. W. & Hendrix, C. E. Hippocampal slow waves: distribution and phase relationships in the course of approach learning. American Medical Association Archives of Neurology, 1960, 3, 74-90.

Adey, W. R., Merrillees, N. C. R. & Sutherland, S. The entorhinal area; behavioral, evoked potential and histological studies of its interrelationships with brainstem regions. Brain, 1956, 75, 414-439.

Adey, W. R., Walter, D. O. & Lindsley, D. F. Subthalamic lesions. Effects on learned behaviors and correlated hippocampal and subcortical slow wave activity. Archives of Neurology (Chicago), 1962, 6, 194-207.

Akert, K. Diencephalon. In D. E. Sheer (Ed.), Electrical Stimulation of the Brain. Austin: University of Texas Press, 1961, 288-230.

Akert, K. & Hummel, P. The Limbic System Anatomy and Physiology. Basle: Hoffmann-La Roche, 1968.

Allen, W. F. Effect of ablating the frontal lobes, hippocampi, and occipito-parieto-temporal (expecting piriform areas) lobes on positive and negative olfactory conditioned reflexes. American Journal of Physiology, 1940, 128, 754-771.

Andersen, P., Eccles, J. C. & Løyning, Y. Recurrent inhibition in the hippocampus with identification of the inhibitory cell and its synapses. Nature, 1963, 198, 540.

Baran, D. & Glickman, S. E. "Territorial marking" in the Mongolian gerbil: a study of sensory control and function. Journal of Comparative and Physiological Psychology, 1970, 2, 237-245.

Bard, P. & Macht, M. B. The behavior of chronically decerebrate cats. In G. E. W. Wolstenholm and Cecilia M. O'Connor (Eds.), Ciba Foundation Symposium on Neurological Basis of Behavior. London: J. and A. Churchill, 1958, 55-71.

Bennett, T. L. Hippocampal EEG correlates of behavior. Electroencephalography and Clinical Neurophysiology, 1970, 28, 17-23.

Black, A. H., Young, G. A. & Batenchuk, C. Avoidance training of hippocampal theta waves in Flaxedilized dogs and its relation to skeletal movement. Journal of Comparative and Physiological Psychology, 1970, 70, 15-24.

Blackstad, T. W., Brink, D., Hem, J. & Jeune, B. Distribution of hippocampal mossy fibers in the rat. An experimental study with silver impregnation methods. Journal of Comparative Neurology, 1970, 138, 433-450.

Bland, B. H. & Vanderwolf, C. H. Effects of hypothalamic stimulation on behavior and hippocampal EEG. Presented to Eastern Psychological Association, Atlantic City, N. J., April, 1970a.

Bland, B. H. & Vanderwolf, C. H. Effects of dentate gyrus stimulation on behavior and hippocampal electrical activity. Proceedings of the Canadian Federation of Biological Societies, 1970b, 13, 47.

Brady, J. V. & Nauta, W. J. H. Subcortical mechanisms in emotional behavior: affective changes following septal forebrain lesions in the albino rat. Journal of Comparative and Physiological Psychology, 1953, 46, 339-346.

Bremner, F. J. Hippocampal activity during avoidance behavior in the rat. Journal of Comparative and Physiological Psychology, 1964, 58, 16-22.

Bremner, F. J. Hippocampal electrical activity during classical conditioning. Journal of Comparative and Physiological Psychology, 1968, 66, 35-39.

Bremner, F. J. The effect of habituation and conditioning trials on hippocampal EEG. Psychonomic Science, 1970, 18, 181-182.

Bremner, F. J. & Ford, J. G. Habituation of neuroelectrical responses negative results. Neuropsychologia, 1968, 6, 65-72.

Brown, Barbara B. Frequency and phase of hippocampal theta activity in the spontaneously behaving cat. Electroencephalography and Clinical Neurophysiology, 1968, 24, 53-62.

Cajal, S. R. Y. Studies on the cerebral cortex. Vol. II Limbic structures. (Trans. by L. M. Kraft), Chicago: Year Book, 1955.

Chatfield, P. O., Battista, A. F., Lyman, C. P. & Garcia, J. P. Effects of cooling on nerve conduction in a hibernator (golden hamster) and non-hibernator (albino rat). American Journal of Physiology, 1948, 155, 179-185.

Corazza, R. & Parmeggiani, P. L. Central course of the afferent system eliciting the theta rhythm upon sciatic stimulation. Helvetica Physiologica et Pharmacologica Acta, 1963, 21, C10-C12.

Crosby, E. C., Humphrey, T. & Lauer, E. W. Correlative Anatomy of the Nervous System. New York: MacMillan, 1962.

Dalton, A. & Black, A. H. Hippocampal electrical activity during the operant conditioning of movement and refraining from movement. Communications in Behavioral Biology, 1968, 2, 267-273.

Delgado, J. M. R. & Sevillano, M. Evolution of repeated hippocampal seizures in the cat. Electroencephalography and Clinical Neurophysiology, 1961, 13, 722-733.

Douglas, R. J. The hippocampus and behavior. Psychological Bulletin, 1967, 67, 416-442.

Eccles, J. C. The Physiology of Synapses. Berlin: Springer, 1964.

Eidelberg, E., White, J. C. & Brazier, M. A. B. The hippocampal arousal pattern in rabbits. Experimental Neurology, 1959, 1, 483-490.

Elazar, Z. & Adey, W. R. Spectral analysis of low frequency components in the electrical activity of the hippocampus during learning. Electroencephalography and Clinical Neurophysiology, 1967, 23, 225-240.

Euler, C. von & Green, J. D. Excitation, inhibition and rhythmical activity in hippocampal pyramidal cells in rabbits. Acta Physiologica Scandanavia, 1960, 48, 110-125.

Flynn, J. P. & Wasman, M. Learning and cortically evoked movement during propagated hippocampal after discharges. Science, 1960, 131, 1607-1608.

Ford, J. G., Bremner, F. J. & Richie, W. R. The effect of hours of food deprivation on hippocampal theta rhythm. Neuropsychologia, 1970, 8, 65-73.

Fujita, Y. & Sato, T. Intracellular records from hippocampal pyramidal cells in rabbit during theta rhythm activity. Journal of Neurophysiology, 1964, 27, 1011-1025.

Gasser, H. S. Nerve activity as modified by temperature changes. American Journal of Physiology, 1931, 97, 254-270.

Gaustaut, H. & Lammers, H. J. Anatomie du rhinencéphale. In: Les Grandes Activités du Rhinencephale. Paris: Masson et Cie, 1960.

Gergen, J. A. Functional properties of the hippocampus in the sub-primate. In W. R. Adey and T. Tokizane (Eds.), Progress in Brain Research. Structure and Function of the Limbic System. Amsterdam: Elsevier, 1967, 27, 442-461.

Gerrard, R. W., Marshall, W. H. & Saul, L. J. Electrical activity in the cat's brain. Archives of Neurology and Psychiatry, 1936, 36, 675-738.

Glickman, S. E., Higgins, T. & Isaacson, R. L. Some effects of hippo-campal lesions on the behavior of Mongolian gerbils. Physiology and Behavior, 1970, In press.

Gogolák, G., Petsche, H., Sterv, J. & Stumpf, Ch. Septum cell activity in rabbit under reticular stimulation. Brain Research, 1967, 5, 508-510.

Grastyán, E. The hippocampus and higher nervous activity. In M. A. B. Brazier (Ed.), Central Nervous System and Behavior. New York: Josia Macy Foundation, 1959.

Grastyán, E. & Karmos, G. A study of a possible dreaming mechanism in the cat. Acta Physiologica Academiae Scientarum Hungaricae, 1961, 20, 41-50.

Grastyán, E., Karmos, G., Vereczkey, L. & Kellényi, L. The hippocampal electrical correlates of the homeostatic regulation of motivation. Electroencephalography and Clinical Neurophysiology, 1966, 21, 34-53.

Grastyán, E., Lissák, K., Madarász, I., & Donhoffer, H. Hippocampal electrical activity during the development of conditioned reflexes. Electroencephalography and Clinical Neurophysiology, 1959, 11, 409-430.

Gray, J. Animal Locomotion. New York: Norton, 1968.

Gray, J. A. & Ball, G. G. Frequency-specific relation between hippocampal theta rhythm, behavior, and amobarbital action. Science, 1970, 168, 1246-1248.

Green, J. D. The hippocampus. In J. Field, H. Magoun and V. E. Hall (Eds.), Handbook of Physiology I. Neurophysiology, Baltimore: Williams and Wilkins, 1960, 2, 1373-1389.

Green, J. D. The hippocampus. Physiological Reviews, 1964, 44, 561-608.

Green, J. D. & Arduini, A. A. Hippocampal electrical activity in arousal. Journal of Neurophysiology, 1954, 17, 533-557.

Green, J. D., Maxwell, D. S., Schindler, W. J. & Stumpf, C. Rabbit EEG "theta" rhythm: its anatomical source and relation to activity in single neurons. Journal of Neurophysiology, 1960, 23, 403-420.

Griffiths, E., Chapman, N., and Campbell, D. An apparatus for detecting and monitoring movement. American Journal of Psychology, 1967, 80, 438-441.

Holmes, J. E. & Adey, W. R. The electrical activity of the entorhinal cortex during conditioned behavior. American Journal of Physiology, 1960, 199, 741-744.

Hopkins, D. A. The neural basis of grooming in the rat. Unpublished Ph.D. thesis, McMaster University, Hamilton, Ontario, 1970.

Ito, M. Hippocampal electrical correlates of self-stimulation in the rat. Electroencephalography and Clinical Neurophysiology, 1966, 21, 261-268.

Jouvet, M. Paradoxical sleep. A study of its nature and mechanisms. In K. Akert, Ch. Bally, and J. P. Schadé. Progress in Brain Research. Sleep Mechanisms. Amsterdam: Elsevier, 1965, 18, 20-57.

Jouvet, M. Mechanisms of the states of sleep: a neuropharmacological approach. Research Publications, Association for Research in Nervous and Mental Disease, 1967, 45, 86-126.

Jouvet, M. & Michel, F. Déclenchment de la "phase paradoxale" du sommeil par stimulation du tone cérébral chez le chat intact et mésencéphalique cronique. Comptes Rendus des Seances de la Societé de Biologic, 1960, 154, 636-641.

Jouvet, M., Michel, F. & Courjon, J. Sur un stade d'activité électrique cérébral rapide au cours du sommeil physioligique. Comptes Rendus des Seances de la Societe de Biologic, 1959, 153, 1024-1028.

Jung, R. & Kornmüller, A. E. Eine Methodik der Ableitung lokalisierter Potentialschwankungen aus subcorticalen Hirngebieten. Archiv für Psychiatrie, 1939, 109, 1-30.

Kandel, E. R. & Spencer, W. A. & Brinley, F. J. Jr. Electrophysiology of hippocampal neurons. I. Sequential invasion and synaptic organization. Journal of Neurophysiology, 1961, 24, 225-242.

Kandel, E. R. & Spencer, W. A. Electrophysiology of hippocampal neurons. II. After potentials and repeditive firing. Journal of Neurophysiology, 1961, 24, 243-259.

Karmos, G. & Grastyan, E. An electrophysiological study of sleep. Electroencephalography and Clinical Neurophysiology, 1960, 12, 933.

Kawamura, H. & Domino, E. F. Hippocampal slow ("arousal") wave activation in the rostral midbrain transected cat. Electroencephalography and Clinical Neurophysiology, 1968, 25, 471-480.

Kawamura, H., Nakamura, Y. & Tokizane, T. Effect of acute brain lesions on the electrical activities of the limbic system and neocortex. Japanese Journal of Physiology, 1961, 11, 564-575.

Kim, C., Choi, H., Kim, J. K., Chang, H. K., Park, R. S. & Kang, L. Y. General behavioral activity and its component patterns in hippocampectomized rats. Brain Research, 1970, 19, 379-394.

Kimble, D. P. The effects of bilateral hippocampal lesions in rats. Journal of Comparative and Physiological Psychology, 1963, 56, 273-283.

Kimble, D. P. The hippocampus and internal inhibition. Psychological Bulletin, 1968, 70, 285-295.

Kimble, D. P. Possible inhibitory functions of the hippocampus. Neuropsychologia, 1969, 7, 235-244.

Kimble, D. P. & Pribram, K. H. Hippocampectomy and behavior sequences. Science, 1963, 139, 824-825.

Kiyono, S., Kawamoto, T., Sakakura, H., & Iwamura, K. Effects of sleep deprivation upon the paradoxical phase of sleep in cats. Electroencephalography and Clinical Neurophysiology, 1965, 19, 34-40.

Kleitman, N. Sleep and Wakefulness. Chicago: University of Chicago Press, 1963.

Klemm, W. R. Electroencephalographic-behavioral dissociations during animal hypnosis. Electroencephalography and Clinical Neurophysiology, 1966, 21, 365-372.

Klüver, H. & Bucy, P. C. Preliminary analysis of the temporal lobes in monkey. Archives of Neurology and Psychiatry, 1939, 42, 979-1000.

Komisaruk, B. R. Synchrony between limbic system theta activity and rhythmical behaviors in rats. Journal of Comparative and Physiological Psychology, 1970, 70, 482-492.

König, J. F. R. & Klippel, R. A. The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem. Baltimore: Williams and Wilkins, 1963.

Kramis, R. C., & Routtenberg, A. Rewarding brain stimulation, hippocampal activity, and foot-stomping in the gerbil. Physiology and Behavior, 1969, 4, 7-11.

Kuehn, R. E. & Zucker, I. Reproductive behavior in the Mongolian gerbil "Meriones unguiculatus". Journal of Comparative and Physiological Psychology, 1968, 66, 747-752.

Lopes da Silva, F. H. & Kamp, A. Hippocampal theta frequency shifts and operant behavior. Electroencephalography and Clinical Neurophysiology, 1969, 26, 133-143.

Lorente de Nó, R. Studies on the structure of the cerebral cortex. I. The area entorhinalis. Journal of Psychology and Neurology, 1933, 45, 381-438.

Lorente de Nó, R. Studies on the structure of the cerebral cortex. II. Continuation of the study of the ammonic system. Journal of Psychology and Neurology, 1934, 46, 113-177.

MacLean, P. D. Psychosomatic disease and the "visceral brain". Recent developments bearing on the Papez theory of emotion. Psychosomatic Medicine, 1949, 11, 338-353.

MacLean, P. D. Chemical and electrical stimulation of hippocampus in unrestrained animals. Part II. Behavioral findings. Archives of Neurology and Psychiatry, 1957, 78, 128-142.

MacLean, P. D., Flanigan, S., Flynn, J. P., Kim, C. & Stevens, J. R. Hippocampal function: tentative correlations of conditioning, EEG, drug, and radioautographic studies. Yale Journal of Biology and Medicine, 1955-56, 28, 380-395.

Milner, B. Amnesia following operation on the temporal lobes. In C. W. M. Whitty and O. L. Zangwill (Eds.), Amnesia, New York: Appleton-Century-Crofts, 1966, 109-133.

Milner, B. & Penfield, W. The effect of hippocampal lesions on recent memory. Transactions of the American Neurological Association, 1955, 42-48.

Moruzzi, G. & Magoun, H. W. Brain stem reticular formation and activation of the EEG. Electroencephalography and Clinical Neurophysiology, 1949, 1, 455-473.

Okuma, T. & Haruo, A. Fundamental and clinical studies on the neural mechanism of sleep. In T. Tokizane and J. P. Schade (Eds.), Progress in Brain Research, Correlative Neurosciences: Clinical Studies. Amsterdam: Elsevier, 1966, 21B, 208-209.

Papez, J. W. A proposed mechanism of emotion. Archives of Neurology and Psychiatry, 1937, 38, 725-743.

Parmeggiani, P. L. On the functional significance of the hippocampal rhythm. In W. R. Adey and T. Tokizane (Eds.), Progress in Brain Research. Structure and Function of the Limbic System. Amsterdam: Elsevier, 1967, 27, 413-441.

Parmeggiani, P. L. & Zannoco, G. A study on the bioelectrical rhythms of cortical and subcortical structures during activated sleep. Archives italiens de biologie, 1963, 101, 385-412.

Paxinos, G. & Bindra, D. Rewarding intracranial stimulation, movement, and the hippocampal theta rhythm. Physiology and Behavior, 1970, 5, 227-231.

Penfield, W. Mechanisms of voluntary movement. Brain, 1954, 77, 1-17.

Petsche, H. & Stumpf, Ch. Topographic and toposcopic study of origin and spread of the reticular synchronized arousal pattern in the rabbit. Electroencephalography and Clinical Neurophysiology, 1960, 12, 589-600.

Petsche, H., Stumpf, Ch. & Gogolák, G. The significance of the rabbit's septum as a relay station between the midbrain and the hippocampus. I. The control of hippocampal arousal activity by the septum cells. Electroencephalography and Clinical Neurophysiology, 1962, 14, 202-211.

Pickenhain, L. & Klingberg, F. Hippocampal slow wave activity as a correlate of basic behavioral mechanisms in the rat. In W. R. Adey and T. Tokizane (Eds.), Progress in Brain Research. Structure and Function of the Limbic System. Amsterdam: Elsevier, 1967, 27, 218-227.

Pompeiano, O. The neurophysiological mechanisms of the postural and motor events during desynchronized sleep. Research Publications, Association for Research in Nervous and Mental Disease, 1967, 45, 351-423.

Pribram, K. H. & Kruger, L. Functions of the "olfactory brain". New York Academy of Sciences, 1954, 58, 109-138.

Raisman, G. The connexions of the septum. Brain, 1966, 89, 317-348.

Rhodes, J. M. Electrical activity of the brain as a reflection of inhibitory phenomena. Neuropsychologia, 1969, 7, 209-216.

Routtenberg, A. Hippocampal correlates of consummatory and observed behavior. Physiology and Behavior, 1968, 3, 533-535.

Routtenberg, A. & Kramis, R. C. Hippocampal correlates of aversive midbrain stimulation. Science, 1968, 160, 1363-1365.

Sailer, S. & Stumpf, Ch. Beeinflussbarkeit der rhinencephalen Tatigkeit des Kininchens. Naunyn-Schmiedelberg's Archiv für die Experimentelle Pathologie und Pharmakologie, 1957, 231, 63-77.

Sainsbury, R. S. Hippocampal activity during natural behavior in the guinea pig. Physiology and Behavior, 1970, 5, 317-324.

Saul, L. J. & Davis, H. Action currents in the central nervous system. Archives of Neurology and Psychiatry, 1933, 29, 225-259.

Siegal, P. S. & Steinberg, M. Activity levels as a function of hunger. Journal of Comparative and Physiological Psychology, 1949, 42, 413-416.

Spencer, W. A. & Kandel, E. R. Hippocampal neuron responses in relation to normal and abnormal function. In P. Passouant (Ed.), Physiologie de l'hippocampe. Paris: Éditions du Centre National de la Recherche Scientifique, 1962, 71-103.

Storm van Leeuwen, W., Kamp, A., Kok, M. L. & Tielen, A. M. Relations between behavior in dogs and electrical activities in various parts of the brain. In E. A. Asratyan (Ed.), Progress in Brain Research. Brain Reflexes. Amsterdam; Elsevier, 1967, 22, 181-194.

Stumpf, Ch. Drug action on the electrical activity of the hippocampus. International Review of Neurobiology, 1965a, 8, 77-138.

Stumpf, Ch. The fast component in the electrical activity of the rabbits' hippocampus. Electroencephalography and Clinical Neurophysiology, 1965b, 18, 477-486.

Stumpf, Ch., Petsche, H. & Gogolák, G. The significance of the rabbit's septum as a relay station between the midbrain and the hippocampus. II. The differential influence of drugs upon both the septal cell firing pattern and the hippocampal theta activity. Electroencephalography and Clinical Neurophysiology, 1962, 14, 212-219.

Swann, H. G. The function of the brain in olfaction. Journal of Comparative Neurology, 1934, 59, 175-202.

Taylor, J. Selected writings of John Hughlings Jackson. London: Staples Press, 1958.

Tolman, E. C. Cognitive maps in rats and men. Psychological Review, 1948, 55, 189-208.

Torii, S. Two types of pattern of hippocampal electrical activity induced by stimulation of hypothalamus and surrounding parts of rabbits' brain. Japanese Journal of Physiology, 1961, 11, 147-157.

Vanderwolf, C. H. Behavioral correlates of "theta" waves. Proceedings of the Canadian Federation of Biological Societies, 1967, 10, 41-42.

Vanderwolf, C. H. Hippocampal electrical activity and voluntary movement in the rat. Electroencephalography and Clinical Neurophysiology, 1969a, 26, 407-418.

Vanderwolf, C. H. Limbic-diencephalic relation in the higher level control of movement. Research Bulletin No. 126, University of Western Ontario, London, Ontario, 1969b.

Vanderwolf, C. H. & Heron, W. Electroencephalographic waves with voluntary movement: study in the rat. American Medical Association Archives of Neurology and Psychiatry, 1964, 11, 379-384.

Wang, G. H. & Akert, K. Behavior and reflexes of chronic striatal cats. Archives of Italian Biology, 1962, 100, 48-85.

Weiss, T. Effect of body temperature changes on theta rhythm in the rat hippocampus. Physiologica Bohemoslovenica, 1964, 13, 246-255.

Whishaw, I. Q. The influence of body temperature on hippocampal electrical activity in freely moving rats. Presented to Eastern Psychological Association, Atlantic City, N. J., April, 1970.

Woods, J. W. Behavior of chronic decerebrate rats. Journal of Neurophysiology, 1964, 27, 634-644.

Yokota, T. & Fujimori, B. Effects of brain-stem stimulation upon hippocampal electrical activity, somatomotor reflexes and autonomic functions. Electroencephalography and Clinical Neurophysiology, 1964, 16, 375-383.

Yoshii, N., Shimokochi, M., Miyamoto, K. & Ito, M. Studies on the neural basis of behavior by continuous frequency analysis of EEG. In T. Tokizane and J. P. Schadé (Eds.), Progress in Brain Research. Correlative Neurosciences: Fundamental Mechanisms. Amsterdam: Elsevier, 1966, 21a, 217-250.