

## HIPPOCAMPAL ELECTRICAL ACTIVITY AND BEHAVIOR IN THE RABBIT

G. FONTANI AND G. CARLI

*Istituto di Fisiologia Umana, Università di Siena, Via A. Moro, 8 I-53100 Siena, Italy*

### INTRODUCTION

Since the earliest studies a close relationship between the hippocampus, in particular its electrical activity, and behavior has been postulated (1, 59, 67). The hippocampal electroencephalogram (EEG) recorded from lower mammals, such as the rat, rabbit or cat, shows a particular slow sinusoidal rhythm named Rhythmic Slow Activity (RSA) or Theta rhythm (56, 83, 92, 110, 116). Another aspect of the hippocampal EEG is an irregular activity of large or small amplitude (116). Studies concerned with hippocampal involvement in behavior and memory functions (66, 81, 93, 107, 109) have revealed the relationship between the hippocampal electrical activity and behavior in laboratory animals in several situations involving arousal (59), orienting response (55), attention (7, 70), learning (2) and voluntary movements (84, 113). Hippocampal EEG patterns, mainly studied by visual inspection of polygraphic recordings, were reported to occur during different types of behavior (11, 48, 97). RSA occurs during voluntary movements, REM sleep and spontaneous immobility (55, 60, 97, 104, 114, 124), but interspecies differences have been described (97, 127). For instance, while RSA in the rat has been recorded almost exclusively during voluntary movements (116, 125), in the rabbit, low frequency RSA has mainly been recorded during spontaneous immobility (62, 74, 91, 111, 115, 128). RSA also accompanies different pharmacological responses to cholinergic drugs (74, 97). In rats and rabbits, a RSA of high frequency (7-12 Hz; type 1 theta, atropine resistant) has been described during walking, running, swimming and other voluntary behaviors (Type 1 behavior) (11, 13, 100, 101, 113) and a RSA of lower frequency (4-9 Hz; type 2 theta, cholinergic, atropine sensitive) has occasionally been recorded during immobility, licking, chewing and other automatic activities (Type 2 behavior). This type of RSA is more easily activated in the rabbit (11, 16, 122, 123). During type 2 behavior, hippocampal activity is mostly irregular (115).

Even though it can be easily recorded, there are still many controversies about the origin and role of the hippocampal electrical activity, and in particular of RSA. Two points are discussed: the origin of theta activity and its relationship with behavior. With regard to the first point, important experiments have shown that oscillators driving the hippocampal "theta cells" exist outside of the hippocampus, specifically in the septum. This has led to the conclusion that the septum and hippocampus are closely related both anatomically and functionally, and to the

postulation of the existence of only one system: the septo-hippocampal system (116). However, other important questions remain unanswered: for example, does RSA represent an active or inactive state of the hippocampus, and what is the exact role played by different hippocampal cells and related structures in originating the theta rhythm (3, 15, 56, 77, 92, 105)? However, we will not discuss these arguments here since our purpose is to deal with the hippocampus behavior relationship in more detail.

Although the existence of a close correlation between hippocampal electrical activity and behavior is largely accepted (1, 59, 67), controversy arises from the different approach of researchers regarding the association of certain behaviors with the hippocampal electrical activity. The canadian group of Vanderwolf, following the tradition of behaviorism in the footsteps of Watson and Skinner, restricted the legitimate subject of behavioral analysis to certain aspects of behavior, such as the presence of movements or immobility, which we can define as modality of behavior. They stated that RSA is correlated with overt behavior in itself rather than with the environmental circumstances or the psychological processes which might be assumed to cause the behavior (98, 115, 116, 117). For example, locomotion is always accompanied by RSA regardless of whether it occurs in a novel or in a well-known environment. Other approaches (7, 56) have primarily considered behavioral motivation and other functional characteristics. In these laboratory experiments, the influence of internal or external factors on behavior was considered crucial and specific RSA patterns, as well as functionally distinct frequency values, have been correlated; for example, with some common paradigms such as goal-directed behaviors (rewarded or active avoidance) or behavioral inhibition (56).

### *1. Novel and emotional stimuli and the hippocampal EEG.*

Because of these different interpretations of behavior, estimates of the relationship between hippocampal electrical activity and behavior do not coincide and the role played by the hippocampus in behavioral situations involving attention, fear, emotionality and learning remained unclear (28, 56, 92, 116). What seemed particularly interesting to us was that we could answer these questions by developing appropriate experimental approaches. Some methodological criteria arose, such as the choice of the animal species to study. The majority of studies have been performed on rats (5, 92, 113), but we preferred rabbits because of the clear hippocampal activity of this species. RSA in particular, is easier to detect in the immobile rabbit than in the rat (62, 97, 118, 119, 120, 121, 127). This permitted a wider range of correlations between hippocampal activity and behavior and, moreover, allowed us to study the electrical correlates of several different kinds of immobility.

Our first approach was to study a particular type of immobility: animal hypnosis (tonic immobility). This is a transitory immobility induced, in the rabbit, by turning the animal upside down and restraining it until it becomes motionless (20,



71, 96). It is characterized by abolition of righting mechanisms, hypotonia of both flexor and extensor muscles and depression of mono- and polysynaptic reflexes (18, 19, 20). We considered that it was a good approach to the study of the hippocampal EEG-behavior relationship. In fact, it was known that hippocampal ablation prolongs hypnosis (129) and evidence existed that cholinergic modulation (physostigmine, scopolamine) affects, in an opposite way, hypnosis and RSA (63). Moreover, it has been shown that hypnosis is prolonged by fear stimuli (49) and we knew that the septo-hippocampal system is involved in anxiety and fear regulation (58).

From the earliest studies, it has been reported that hippocampal theta rhythm is only occasionally present during animal hypnosis (18, 71, 72, 73, 102, 104). Nevertheless, detailed quantitative studies did not exist. Our aim was not only to record the occurrence of RSA, but to determine, in detail, the temporal distribution, amount and frequency of hippocampal RSA during animal hypnosis, as well as to evaluate the existence of possible relationships between hypnosis characteristics and patterns of hippocampal electrical activity.

The results of this experiment (39) were that the occurrence, duration and frequency of RSA were different in consecutive hypnosis periods and that there was an inverse relationship between hypnosis duration and RSA parameters. Frequency decreased as the rabbit passed from movement to spontaneous immobility, and then from spontaneous to induced immobility (hypnosis). This suggested that the two types of immobility could be discriminated according to theta rhythm frequency distribution. Moreover, data showed that RSA varies according to the period of hypnosis. In particular, it disappears during induction, when the experimenter is still restraining the animal and, during hypnosis, only short-lasting theta episodes are recorded. RSA parameters (number of episodes, total amount, duration of a single episode, mean frequency) are lower in the first period of hypnosis, gradually increasing from the beginning to the end. On the basis of these results, we suggested that the beginning of hypnosis was indicated by the disappearance of theta rhythm during induction procedures. Similarly, the reappearance of RSA of higher frequency, before the onset of the first movement, could indicate that hypnosis was about to end.

The differences in the occurrence and frequency of RSA observed between spontaneous and induced immobility, and within hypnosis periods, led us to the conclusion that different levels of arousal could modulate hippocampal activity during a uniform behavioral situation, such as animal hypnosis. With regard to methods, it should be underlined that the quantitative analysis of RSA parameters, such as number of episodes, total amount, duration of a single episode and mean frequency, was crucial in determining the characteristics of the behavioral conditions studied.

These results encouraged us to extend our interest to other aspects of behavior. As concerns movement, exploratory behavior in animals has been associated with attention and emotionality and a relationship between fear and exploratory activity has also been proposed. Novel stimuli seem to be effective in generating orienting

behavior, exploration and anxiety (56, 106). Behavioral responses elicited by novel stimuli include the inhibition of the ongoing behavior, as well as an increase in arousal and attention. The septo-hippocampal system could be one of the neural structures involved in these reactions. Moreover, the hippocampus seemed to be essential for exploratory behavior. Some theories suggest that the hippocampus has a function in attention and stimulus sampling, and others propose that it plays a role in the regulation of anxiety and fear (7, 53, 56, 78, 94, 103, 126). The effects on hippocampal electrical activity of the presentation of novel stimuli could be useful for an understanding of the relationship between EEG and behavior. In view of these findings, we developed an experimental model based on the presentation of different novel stimuli (36). Natural behaviors that do not involve negative or positive reinforcement were selected in order to minimize motivational factors. Two types of stimuli were presented: immobile and mobile. The immobile stimuli consisted of a novel environment (a moderately lighted, ventilated cage), a novel object, a tin containing vegetable branches (rosemary or cabbage) and a stuffed animal (sparrow hawk), while the mobile stimulus consisted of the introduction to the experimental cage of a live cat.

Different patterns of hippocampal electrical activity were recorded in the presence of these different environmental stimuli. As for RSA, the novel object elicited an increase of the high frequency band during movement. In the presence of the stuffed sparrow hawk, behavioral and electrical activities greatly differed from those recorded in the neutral environment. RSA frequency distribution tended to increase in the low frequency band during both immobility and movement and electrocorticogram (ECoG) synchronization was recorded. The temporal pattern of behavioral responses following the presentation of the stuffed animal was completely different from that observed in the other experimental situations, with a clear reduction of activity. On the whole, these observations suggested that the presentation of inanimate objects resulted in different responses according to the significance of the stimuli. In particular, the presentation of the stuffed animal contained several cues able to release patterns of RSA, ECoG synchronization and behavior typically associated with a low level of arousal. The pattern of response to the stuffed animal showed close similarities with that observed during the immobility induced in the rabbit by inversion (animal hypnosis) (39), where a great amount of ECoG synchronization develops, along with changes in RSA parameters, such as reduction of percentage and frequency. It has been suggested that the response of the animal to hypnosis represents the ultimate defensive reaction to threatening stimuli. By analogy, the complex response to the stuffed animal could be interpreted as a cut-off of the external stimuli triggered by some cues eliciting high emotionality (60, 80).

In the presence of a live cat (36), the amount and frequency of RSA reached high values, particularly when the cat was looking at the rabbit. These results seemed to support the hypothesis of a relationship between RSA and stimulus processing mechanisms. The presentation of the mobile, animate stimulus (cat) corresponded to a strong increase in the amount and frequency of RSA in comparison with the



other experimental situations. Moreover, video-tape recordings showed that high frequency RSA episodes occurred suddenly whenever the cat changed the position of its head or looked at the rabbit.

Another aspect observed was a considerable overlap of frequency spectra recorded during immobility and movements. This led to the conclusion that frequency could be an important discriminative means of determining the rate and nature of information processing in the hippocampus, but that it is not a reliable parameter to distinguish theta and non-theta activity. Also, an inverse correlation was found between the amount of RSA and the percentage of the high frequency band in the presence of the immobile stimuli (35, 36).

## II. A neuro-ethological approach.

The experiments described above suggested that the hippocampal RSA could be differentially related to the nature of the stimuli presented and that emotional and cognitive aspects of behavior could influence hippocampal activity. However, it seemed to us that the association of a single behavioral variable, such as modality (movement, immobility), with a type of electrical activity was simplistic, since behavior and EEG patterns were much more complex. On the other hand, the selection of a few laboratory paradigms risked losing sight of behavior as a whole. Experimental conditions which favor the natural flux of behavior thus seemed most suited to a study of the dynamics of behavior-related hippocampal electrical activity. In fact, ethological techniques have shown that behavioral elements, which are very complex in natural conditions where they can be modified by environmental stimuli (31, 54, 64), need to be considered under different categories and variables (54, 64). Ethology looks for units of behavior which are meaningful patterns in the lives of animals. Many of these units consist of complex behavioral patterns which cannot be isolated from the settings that make them meaningful. For example, from an ethological perspective, there is a difference between running toward a receptive female and the same movement made in getting away from a predator. Moreover, as for EEG, the development of modern techniques, such as power spectrum analysis, has revealed that the frequencies of different hippocampal EEG patterns often overlap (79). This means that it was necessary to redefine EEG patterns and behavior in terms of additional parameters and to develop a neuro-ethological approach (30) in order to collect information about the relationship between behavior and hippocampal activity (36, 41, 65, 79). The use of this approach, applied to both behavior and EEG, could be expected to give more specific electrical correlates of behavior.

First, we classified behavioral elements according to an ethological method and divided them into categories (Tab. I) that corresponded more to the complexity observed in nature (44). Then, we planned experiments in which rabbits were introduced to an enclosure in the open air, bordered on three sides by trees and fields (semi-natural environment) (Fig. 1). The enclosure consisted of a 5x5 m plot

Table I. - Behavioral elements recorded during the experimental observations.

<i>Elements</i>	<i>Modality</i>	<i>Function</i>	<i>Motivation</i>
<b>Category 1: Behavior directed toward the environment</b>			
Explore (Ex)	voluntary mov.	exploration	cognitive
Scan (Sc)	"	"	communicative
Walk (Wk)	"	"	"
Run (Rn)	"	"	"
Mark (Mk)	"	signalling	"
Alert (Al)	immobility	reactive	reactive
Sit (Sj)	"	postural	postural
Crouch (Cr)	"	"	"
<b>Category 2: Self-directed activity</b>			
Shake (Sh)	automatic mov.	autocleaning	inspective
Scratch (Sr)	"	"	"
Wash (Wa)	"	"	"
Self-grooming (Sg)	"	"	"
Eat (Ea)	"	feeding	conservative
Drink (Dk)	"	"	"
<b>Category 3: Social activity</b>			
Point (Pt)	immobility	Interactive	relational
Attend (At)	voluntary mov.	"	"
Approach (Ap)	"	"	"
Retreat (Rt)	"	"	"
Nose (No)	"	"	"
Investigate (In)	"	"	"
Fight (Fh)	"	"	agonistic
Attack (Ak)	"	Offensive	"
Follow (Fo)	"	"	"
Chase (Ch)	"	"	"
Mount (Mt)	"	"	"
Bite (Bi)	"	"	"
Cross-over (Co)	"	"	"
Grooming (Gr)	"	"	"
Flight (Fl)	"	Defensive	"
Submit (Sm)	"	"	"
Freezing (Fr)	immobility	"	"
Subdued posture (Sp)	"	"	"

surrounded by wire netting and furnished with a shelter box and containers for food and water, provided ad libitum. Seven days before the introduction of the animals to the semi-natural environment, they were implanted with four nichrome electrodes (200  $\mu$ m), insulated except at the tip, two in each side of the hippocampus. One electrode was positioned in the stratum oriens of the CA1 pyramidal layer and the other in the dentate molecular layer. At the end of the experiment, the exact

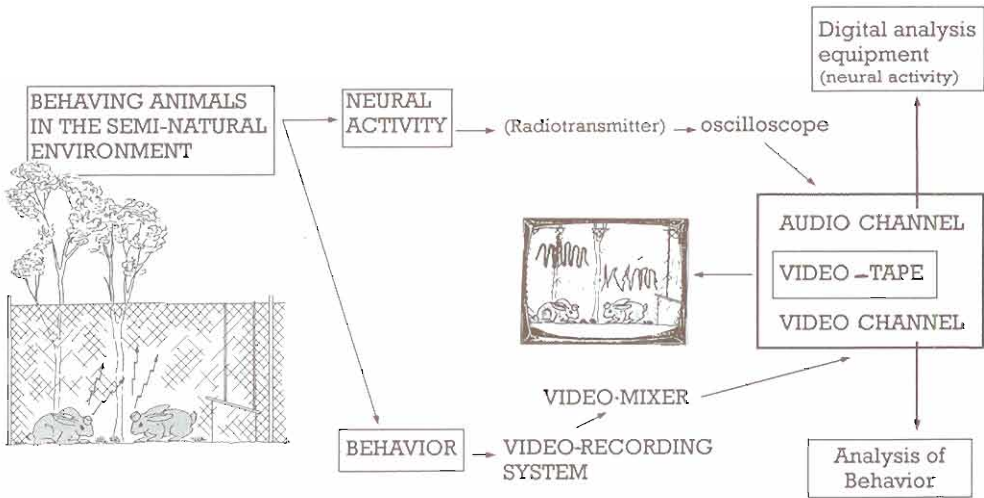


Fig. 1. - *The neuro-ethological experimental approach.*

Electrical activity is recorded by telemetry and behavior by a video system. Both are stored on video-tape: electrical activity on the audiochannel and behavior on the videochannel. This allows the analysis of each behavioral element and of the corresponding electrical correlates.

position of the electrodes was checked using dimethylglyoxime (42). The wires were connected to a 9-contact Amphenol plug and the assembly was cemented with dental acrylic. Neural activity was recorded by radio-telemetry (34, 41). Normal animal behavior was not impaired by the small dimensions and weight of the transmitter. A radio receiver connected to an aerial was located in the laboratory near the enclosure. Signals were filtered by a low-pass filter and then displayed on a multichannel oscilloscope.

Two video-cameras were used to monitor animal behavior (one camera at the window, in front of the enclosure) and electrical activity (a second camera in front of the oscilloscope). The images recorded by the two cameras were displayed on two different TV monitors. They were then mixed by a video-mixer and displayed on a third monitor. In this way, it was possible to record the electrical activity of an animal superimposed on the picture of its behavior. The images displayed by the third monitor were recorded by a stereo video-cassette recorder with two separate audiochannels (Fig.1). The data stored in video-cassette by this method were processed by qualitative and quantitative analysis of the bioelectric data recorded on the audiochannel of the tape. Radio-telemetry and the video-recording techniques enabled the neurophysiological data to be associated directly and accurately with the concomitant behavior (4). In this way, electrical samples could be ascribed to specific behavioral elements (41) and frequency shifts could be observed, for example, during the switch from one behavioral act to another (68). As regards the analysis of electrical data, a digital approach seemed to be more appropriate for studying the complex relationships existing between electrical



activity and behavior (29, 75, 79). Digital analysis and the study of several electrical parameters (power spectra, autocorrelation, standard deviation, S.D., and coefficient of variation, C.V.) allowed an electrical pattern to be assigned on the basis of frequency and rhythmicity to each behavioral element.

We introduced a single rabbit to the semi-natural, open-air environment and observed its behavior and related hippocampal electrical activity for three days. Behavioral items, all nonsocial in nature, were first divided into two broad categories (behavior directed toward the environment and self-directed behavior) (Table I). In each category, behavioral elements were clustered into subcategories according to variables such as modality, function and motivation (Table I). Modality refers to the manifestation of a behavioral element, e.g. immobility or movement; function represents the characteristic action of a group of behavioral elements; motivation refers to the expected purpose of the activity, e.g. cognitive: getting to know the new environment by exploration. Each behavioral item was studied by recording its occurrence and duration and its related hippocampal activity. Each electrical sequence, related to a behavioral element, was divided into samples of 1.2 seconds and then analyzed.

The use of a semi-natural environment allowed the observation of a wide range of behaviors (Table I), occurring naturally and continuously. This differentiates our approach from many laboratory studies in which only a limited number of evoked behavioral elements were taken into account (56, 74, 92, 116). Behavioral and electrophysiological effects occurred after the introduction of a rabbit to a novel semi-natural environment. Some behavioral elements were correlated to specific patterns of hippocampal electrical activity, which could undergo modifications during the experimental period.

On the basis of the electrical activity recorded during each behavioral situation, we could apply specific electrical profiles to the behavioral elements observed in semi-natural conditions. Our results showed that these profiles were characterized by the typical aspects of autocorrelation data, power spectra, S.D. and C.V.. In particular, the analysis of autocorrelation showed that rhythm is a peculiar aspect of hippocampal EEG and allowed a classification of the electrical layout based upon rhythmicity. Three electrical patterns were defined: HRSA (High Rhythmicity Slow Activity) characteristic of certain voluntary elements such as explore and run; LRSA (Low Rhythmicity Slow Activity), recorded in signalling activities or self-directed behavior, as well as during immobility (both postural and reactive); and IA (Irregular Activity), recorded in some instances during the same automatic or immobile behaviors (Fig. 2).

With regard to some behavioral elements already tested in the laboratory experiments, their relationship with hippocampal EEG not only appeared more complex, but they were also enriched with more specific features. For example, exploration showed peculiar characteristics which differentiate it from other behavioral elements and also varied with time, showing effects of adaptation to the environment. The exploration of the novel environment is long-lasting and progressively covers a wider space, the zone initially explored by the animal possibly being limited by



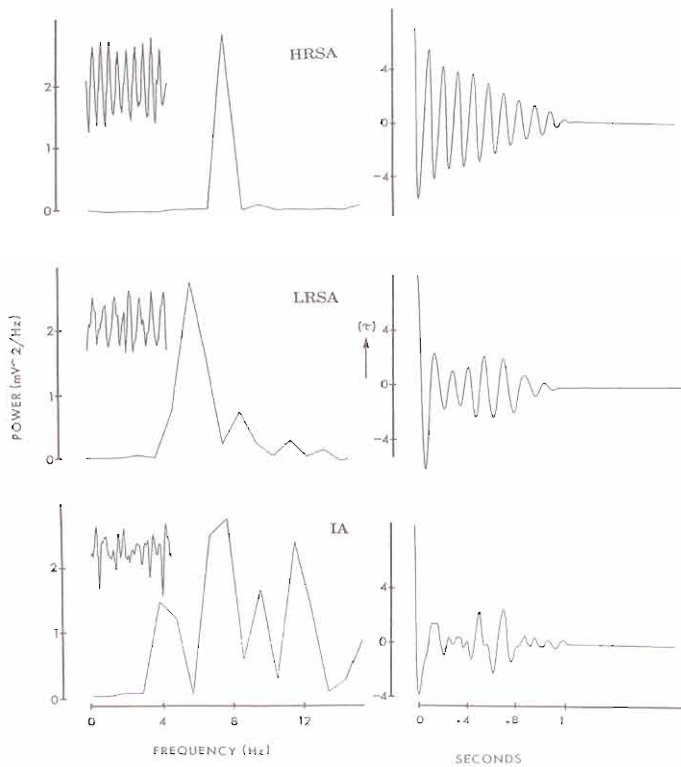


Fig. 2. - The three main patterns recognized in the hippocampal electrical activity according to frequency and rhythmicity.

HRSA (High Rhythmicity Slow Activity), LRSA (Low Rhythmicity Slow Activity) and IA (Irregular Activity). On the left are represented characteristic samples of 1.2 sec periods of hippocampal activity and their power spectra. On the right: their corresponding autocorrelation analysis.

emotionality and fear. Specific frequencies had been associated with exploration in rats (57). In our approach, the rabbits showed a peculiar pattern of hippocampal EEG during exploration; the frequency, concomitant with a reduction in exploratory activity, increased with time (44).

As for immobility (43, 44) and self-directed activities (47), typical electrical differences were observed. Specific patterns of hippocampal EEG differing in S.D. and C.V. could be ascribed to alert and postural immobility, as well as to autocleaning and feeding behavior. Progressively decreasing values of S.D. and C.V. were observed from postural to alert immobility, through self-directed activities to voluntary behavior. Power spectra and autocorrelation data, concomitant with an evident overlap in frequency (Fig. 2), led to the conclusion that hippocampal activity is uniform in character, changing in rhythmicity and frequency according to the strength of the stimulus. It has been reported that the majority of hippocampal cells discharge in a rhythmic manner, closely related to the period of the waves and

locked to the negative phase of RSA (theta cells) (8, 112). These cells discharge irregularly during an irregular hippocampal EEG and have been described as being rhythmically active during type 1 and 2 RSA (12). Situations such as postural immobility, involving neither voluntary activities nor a high degree of processing of specific stimuli, may not affect theta cells, which can continue to discharge in an irregular pattern. A different origin can be ascribed to the IA recorded during alert immobility; it may be related to behavioral inhibition in anticipation of an unknown event, as often happens after noises or other stimuli occurring in the environment outside the enclosure. In other conditions, a large number of theta cells may be activated in phase, evoking RSA which can be either HRSA or LRSA according to the number of theta cells recruited in phase. Cholinergic and non-cholinergic pathways to the hippocampus may be involved in this activation thus leading to the pharmacological differences observed in the origin of RSA (74). Therefore, if hippocampal EEG patterns depend on the modulation of theta cells, the relationship between hippocampal electrical activity and behavior is a consequence of the strength at which a stimulus evoking a behavioral element is able to activate the hippocampus. The neuro-ethological approach shows that natural conditions may influence behavioral and cerebral responses. These responses need to be considered in relation to a continuous flux of environmental information influencing the evolution of behavioral activities, each of them corresponding to a typical electrical pattern depending on modality and functional and motivational aspects.

Particularly important seems to be the role played by anxiogenic and emotional stimuli. A relationship between the hippocampus and emotionality had been widely postulated (36, 50, 57, 100). In the immobile rat, the presence of a cat or a ferret produced RSA (otherwise difficult to elicit) (100) and we described that, in the rabbit, the presentation of a cat was accompanied by a high percentage of RSA of high frequency (36). However, in the studies reported above, the experimental paradigm limited the behavior of the animals and flight was impossible. Ethological studies have shown that, in natural conditions, reactions to threatening stimuli are characterized by three main responses: attack, immobility or flight. Since the last two responses were easily observed reactions to a predator (64), the study of their electrical correlates became crucial. Our purpose was to correlate emotional behavior and hippocampal electrical activity by studying the effects of highly emotional and specific stimuli, such as the presentation of a dog (a predator) (46) or a conspecific (another male rabbit: intruder test) (45), in a natural setting (semi-natural environment) permitting every kind of response. In an experiment described above (36), we reported the effect on hippocampal EEG of the presentation of a cat to a rabbit in a small experimental cage. RSA increased with respect to other patterns and also its frequency was higher. This experiment suggested a relationship between hippocampal EEG and the qualitative aspects of the stimulus. Although the experiment also indicated an increase in RSA frequency, the behavioral responses of the animal were far different from those elicited in natural conditions. A threatening stimulus, such as the appearance of a dog in a natural setting, was

more appropriate to evoke typical behavioral reactions and changes in hippocampal electrical activity. Moreover, the introduction of the dog even influenced the hippocampal EEG in the period after the removal of the threatening stimulus, at least for some behavioral elements.

The electrical activity of the hippocampus appeared to be specifically affected by the nature of the stimulus, the emotional situation of the animal and the defensive strategy adopted: flight or immobility. This can be explained by the differences observed between the electrical correlates of behavioral elements showing the same modality. For example, run and flight are both voluntary movements, but the electrical activity recorded in running during the periods before introduction and after removal of the dog had lower frequency peaks than that recorded during flight in the presence of the dog (46).

### III. *Social interactions.*

Interactions between animals of the same species require complex behavioral items involving a range of reactions, from relational to agonistic elements (64). It has been reported that rabbits, introduced in pairs to a semi-natural environment, show agonist behavior characterized by the prevalence of offensive or defensive reactions, which lead to a dominance-subordination polarity (6, 9, 10, 17, 24, 33, 85, 86, 87, 88, 130). The different behavioral strategies adopted are accompanied by concomitant variations in hormonal and metabolic levels (24, 31).

The use of the intruder test in the semi-natural environment could be useful to confirm the behavioral and neurophysiological modifications previously observed in the dog test and to allow the acquisition of new information about the effects of social activity on the electrical activity of the brain. After a period of adaptation to the environment (six days) of the resident rabbit, a second rabbit (intruder) was released in the enclosure. The intruder was of the same species and sex as the resident. Peculiar behavioral responses were observed in the resident animal. After the first contact (nose), the resident assumed offensive and defensive attitudes toward the intruder. These attitudes led to typical offensive behavior in some residents and defensive behavior in others. The hippocampal electrical activity of the resident underwent modifications in parameters, i.e. in pattern, frequency and variability, connected with the behavioral aspects. Compared with periods in which no direct interactions were observed, relational and agonistic elements showed higher frequency peaks and less variability. Particularly interesting was the observation that different frequency values characterized behavioral elements of similar modality, but different functional and motivational aspects (45). These differences can be observed both in voluntary movements (chase, flight, run) and immobility-related elements (point, alert, freezing). In this approach, the behavioral analysis was not aimed at establishing a clear dominant-subordinate relationship. However, although the analysis was limited to the first period of interaction between resident and intruder, it was still possible to distinguish rabbits in which offensive elements prevailed and other rabbits in which there was a prevalence of



defensive elements (45). These two groups (offensive and defensive rabbits) not only showed particular behavioral aspects but also distinct electrical profiles. In the presence of the intruder, no difference in frequency was observed between offensive and defensive rabbits in the first period of approach, until the contact with the other animal (nose) (76). After nose, animals which responded with defensive reactions were characterized by higher frequency values during their specific conflictual elements (defensive) than offensive rabbits and the frequency was higher in offensive rabbits in some elements (explore, alerting movements) in the period following the removal of the intruder.

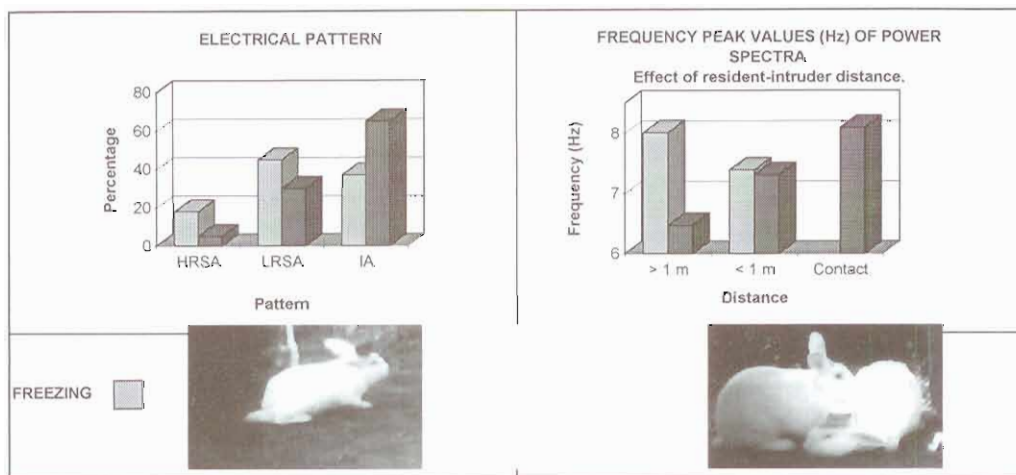


Fig. 3 - Percentage of electrical patterns (left) and frequency peak values (Hz) of power spectra depending on the resident-intruder distance observed during two types of immobility reactions (freezing and subdued posture) in the presence of an intruder in the semi-natural environment.

Particularly close to this point is the observation that different immobility aspects are related to different electrical patterns (43). Two different kinds of immobility were observed (Fig. 3), both being reactions to a threatening stimulus (the presence of the intruder). Freezing (Fr), largely observed in laboratory experiments (108), was preferred when the intruder was exploring at a distance. Subdued posture (Sp) was observed when the intruder was near and interacting with the resident. Hippocampal EEG showed a prevalence of rhythmic activity during Fr and irregular activity during Sp. In the latter, the frequency depended on the intruder behavior and increased with the vicinity and interactions of the intruder (Fig. 3). Moreover, the irregular waves recorded during Sp were similar to those previously recorded in our laboratory in the presence of the stuffed sparrow hawk (36) or during animal hypnosis (39).

Similar effects could be observed during self-directed behavior, which can be defined as all activities directed by the animal toward its own body. These are

characterized by repetitive automatic movements and can be divided into two subcategories: autocleaning (washing and self-grooming) and feeding activities (47, 61). Some of the behavioral elements belonging to these groups such as grooming are reported to increase with novelty (90) in stressful situations (99) and after adrenocorticotrophic hormone (ACTH) administration (25). Comparing self-directed activities in quiet and emotional situations, such as those observed during solitary behavior and during the intruder test, we confirmed the influence of emotional and motivational aspects on the electrical activity recorded during behaviors of the same modality (Fig. 4). This strengthened the hypothesis that a behavioral element is not uniform from a neuro-electric point of view, since hippocampal EEG depends largely on its functional and motivational correlates.

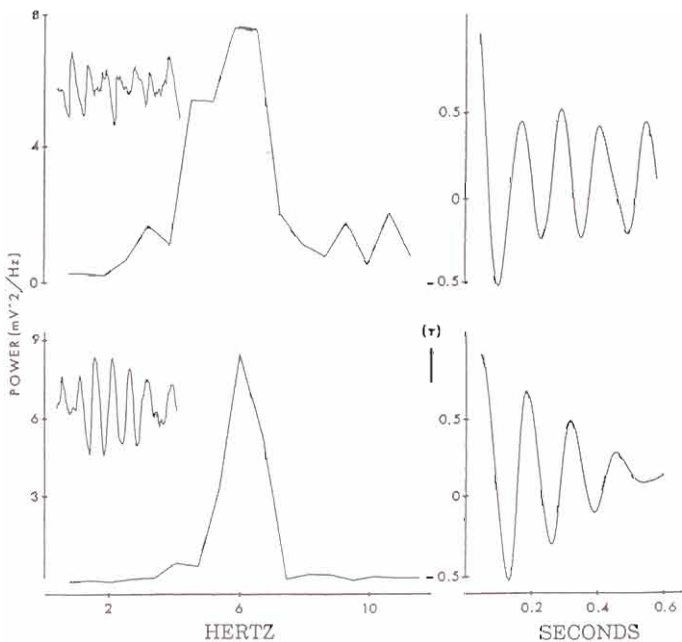


Fig. 4. - *Self-grooming recorded during solitary behavior (top) and in the presence of the intruder (bottom).*

Averaged 1.2 sec samples of hippocampal electrical activity. Left: power spectra; right: autocorrelation analysis.

#### IV. *Dominant and subordinate rabbits.*

The experiments described above showed that the introduction of an intruder of the same species and sex to the semi-natural environment elicited offensive and defensive reactions by the resident (27), which were characterized by different electrical profiles (45). These results, from a short period of social activity,

prompted a more detailed examination of the relationships between social behavior and hippocampal EEG. We then tried to study the long-lasting electrical effects produced in the hippocampus by social activity, recording the hippocampal electrical profiles of the dominant and subordinate animals and analyzing their temporal variations during the establishment of social rank (40).

Two rabbits of the same sex and weight were introduced contemporaneously to the seminatural, open-air environment and their social and non-social behavior and hippocampal EEG were recorded for four days by the same method described above. The activity of each rabbit was recorded contemporaneously by telemetry. At the end of this period, a third rabbit was introduced to the environment and the reactions of the residents were studied for 30 minutes (intruder test). Behaviors were divided into dominant and subordinate elements. The dominant elements, behaviors described previously as positively correlated with dominance (33, 31, 54, 69), were the environment-related behaviors - explore, scan, mark and run; the interactive elements - approach and investigate; and the whole group of offensive behaviors. The subordinate elements, behaviors negatively correlated with dominance (31), were: the defensive behaviors - flight, freezing and subdued posture; and the self-directed activities-self-grooming, wash and scratch. For each pair, it was possible to establish the dominant and subordinate animal since one rabbit clearly exhibited a greater total score of dominant elements and the other one showed more subordinate behavior.

The results of this study show that hippocampal electrical activity is modulated in relation to the behavioral modifications involved in the establishment of a dominance-subordination relationship. In the pairs of rabbits studied, we were always able to identify a dominant and subordinate animal, even though the animals seemed to start with equal possibilities of developing dominance or subordination. The introduction to the semi-natural environment elicited agonistic reactions, with behavioral differentiation evident already in the first two observations. In the following periods, interactions and general activity were reduced and dominant and subordinate elements did not clearly differentiate the two animals of the pair. However, reversals of the social rank were never observed and the intruder test confirmed the previous attribution of dominance.

Hippocampal electrical activity showed higher levels of frequency in dominant rabbits than in subordinate ones, at least for behaviors such as crouch, explore, nose and investigate (Table II). Moreover, with regard to the distribution of frequencies (power spectra), recorded for each episode of 1.2 seconds and divided into nine frequency bands, there was an increase of the high frequency bands (from 9.2 to 15 Hz) from observation 1 to 4 in the dominant animals. This was observed for some of the most prominent behavioral elements, such as crouch, nose or fight, and other behaviors related to dominance, such as explore, approach and investigate. For behavioral elements related to subordination (subdued posture and self-grooming), a clear increase of high frequency bands was observed in subordinate rabbits.

This approach suggests that dominant rabbits, in this experimental situation and



Table II. - Mean frequency peak values (Hz) of power spectra of hippocampal electrical activity recorded during social interactions in the more frequent behavioral elements.

Behavioral elements	Dominant rabbits	Subordinate rabbits	Significance*
Crouch	8.711	7.580	P<0.02
Explore	7.938	7.460	P<0.02
Nose	8.158	7.278	P<0.05
Investigate	8.024	7.601	P<0.02
Fight	9.020	9.350	N.S.
Subdued posture	7.472	6.898	N.S.

\* Paired Samples t-test

in the presence of specific behavioral elements, develop increases of EEG frequency and rhythmicity. However, the increase of frequency is not immediate. In fact, it was not observed in the first observation, but was particularly evident in the third and fourth sessions. Therefore, the variations in electrical activity seem to appear later than the behavioral differentiation, which was particularly evident in the first and second observations.

This delay can be explained on the basis of hormonal responses since it has been reported that, concomitant with the establishment of a dominance-subordination relationship, there are modifications of hormonal parameters (31, 32, 33, 52). In particular, testosterone and estradiol have been associated with dominance and corticosterone, ACTH and androstenedione with subordination (31). Moreover, these hormonal responses, and also metabolic (21, 23, 51) and immunological ones (52), do not seem to be immediate, but represent a longer-lasting adaptive mechanism accompanying the establishment of social rank (31).

A wide range of corticosteroid receptors has been described in the hippocampus (14, 82, 89) and corticosterone is known to influence the electrical activity of pyramidal neurons (22, 37, 95), as well as to modulate hippocampal cholinergic activity (26, 51). In a previous paper, we described that ACTH (1-24) decreased high frequency bands and increased low frequency ones (38). On the basis of these results, we can assume that lower levels of corticosteroids could be related to higher frequency values of hippocampal EEG in the dominant rabbits and higher levels of corticosteroids in the subordinate rabbits cause the lack of increase in frequency levels in those animals.

## CONCLUSIONS

The neuro-ethological approach has shown that the relationship between hippocampal electrical activity and behavior cannot be reduced to simple movement-immobility correlations, but involves complex aspects of behavior characterized by emotional, attentional and cognitive aspects. The latest studies on social interactions have demonstrated the possibility of recognizing electrical profiles of

aspects related to social rank and have assigned to frequency and rhythmicity the role of crucial parameters able to reveal dominant and subordinate attitudes. The hippocampus seems to have an important function in modulating the establishment of behavioral items involved in complex reactions to environmental stimuli. This role depends upon hormonal and metabolic influences and can be observed by studying the variations of hippocampal EEG parameters occurring during behavioral changes in natural or semi-natural conditions.

#### SUMMARY

Different approaches to the study of the relationship between hippocampal electrical activity and behavior have shown that hippocampal EEG varies according to different behavioral aspects. In particular, a rhythmic slow activity (RSA, Theta rhythm) can be recorded during voluntary movements and to a lesser degree during immobility. In laboratory experiments, we tried to evaluate the influence on the hippocampal EEG of external stimuli evoking emotional or cognitive responses. Similar electrical responses were observed during animal hypnosis and in the presence of threatening stimuli, while some electrical parameters, such as frequency and rhythmicity, varied according to the nature of the stimulus. In order to have greater and clearer information, we adopted a neuro-ethological approach, which allowed us to classify behavior into several categories and to record the hippocampal EEG during natural behavior. The influence exerted on hippocampal electrical activity by novel stimuli and the electrical profiles recorded during exploratory behavior or alert or quiet immobility allowed us to classify the hippocampal EEG on the basis of frequency and rhythmicity. Moreover, the study of social behavior showed that the variations of the electrical parameters of the hippocampal activity depended on the characteristics of the stimulus and that typical electrical profiles could be observed in immobile or motor activities caused by specific stimuli. Socially dominant and subordinate rabbits could also be distinguished on the basis of hippocampal electrical parameters.

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