HIPPOCAMPAL ELECTRICAL ACTIVITY AND DIFFERENT TYPES OF CONDITIONED REFLEXES IN DOGS

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Abstract. Changes in hippocampal activity and heart rate were studied in dogs during elaboration of defensive and alimentary conditioned reflexes and switching-over between different types of reinforcement. Conditional stimuli enhanced synchronization and increased frequency of theta wave trains in hippocampal electrical activity. Parallelly to emotional tension of the dogs a significant rise in amplitude of integrated theta rhythm appeared. An increase in the positive correlation between the changes in amplitude of integrated theta rhythm and in heart rate shows that the hippocampal theta rhythm may be used as a correlate of emotional tension. Changes in hippocampal electrical activity in the early stages of formation of conditioned connections and throughout the experiment with conditioned "switching-over" suggest that emotional tension arises under conditions of pragmatic uncertainty when the animal does not have sufficient information for the organization of adaptive reactions. More marked changes in heart rate and in amplitude of hippocampal theta rhythm during the elaboration of defensive CRs compared with those observed during the development of response eliminating nociceptive stimulation of partner, suggest that the magnitude of emotional tension depends on the conditioned excitation underlying the response being elaborated.

INTRODUCTION

By recording hippocampal electrical activity under a variety of experimental conditions, characteristic trains of regular, slow waves in the theta rhythm range have been observed. There is no agreement in the literature as to the behavioral correlates of the hippocampal theta rhythm. Theta rhythm has been observed during the elaboration of conditioned reflexes (1, 5, 7, 9); during the orienting reflex (7, 19); in a state of at-
tention (4); during the performance of so-called “voluntary” movements (23) or goal-directed behavior (8); and during tension under stress (2). Some of the foregoing responses are known to have emotional accom-
paniment.

The present study was thus designed to explore the relation, between appearance of hippocampal synchronization and emotional stress. Change in heart and respiratory rates were recorded together with electrical activity of the hippocampus. Since, according to Pavlov, the first phase of conditioning associated with a change in the animal’s behavior involves emotional elements, the study was limited mainly to the early phase of conditioning.

METHODS

Hippocampal electrical activity and heart rate were recorded in dogs during the development of defensive and alimentary conditioned reflexes, during conditioned reflex “switching-over” to heterogeneous reflexes, and during aversive electrical stimulation of another dog.

Preliminarily, under aseptic conditions, electrodes were implanted in different hippocampal regions using stereotaxic coordinates (12). Recording was done on a 17-channel Nihon-Kohden encephalograph with a two-channel band analyzer for frequency and a frequency integrator. Changes in amplitude of the integrated theta rhythm were evaluated statistically, the significance of differences being determined from Student’s test. The correlation coefficient was calculated between the amplitude of the integrated theta rhythm and heart rate measured during the same time period.

From the first pairing the conditional stimulus (CS) was switched on 10 sec before the beginning of unconditional stimulation (US), since the epoch of accumulation of electrical activity by the integrator was equal to that time period.

After the experiment, a morphological control was run to confirm the localization of electrode tips.

RESULTS

In preliminary tests, the percentage of different frequencies (in the range from 2 to 30 Hz) was determined in leads from the dorsal hippocampus. Under these conditions, the hippocampal electrical activity was dominated by slow wave trains (delta and theta waves) which constituted some 60–65% of the total power of the spectrum.
In five dogs, changes in hippocampal electrical activity were studied during the elaboration of instrumental defensive reflexes and during the switching-over (3) to different (alimentary and defensive) conditioned reflexes (CRs). The results obtained during the development of defensive CRs and of defensive responses in switching-over were similar and so will be considered together.

The animals showed strong behavioral changes after only a few trials with defensive CRs. They refused to enter the chamber, tried to bite off the wires, and get rid of the cuff and lever which were fixed to their paw. During that period, all dogs exhibited marked autonomic changes, including increased cardiac and respiratory rates. Pairing of acoustic (CS) and electrodermal (US) stimuli began to elicit changes in electrical activity of the dorsal hippocampus after several presentations. As can be seen from Fig. 1, exposure to the defensive CS elicited trains of

![Fig. 1. Action of conditioned defensive stimulus. 1, 2, hippocampal EEG; 3, EKG; 4, respiration; 5-7, delta, theta and alpha cycles as isolated from EEG by analyser; 8, total power of hippocampal EEG; 9, paw lifting; 10, application of shock; 11, application of CS. Dog Pyatnashka.](image)

synchronized slow waves in the theta-rhythm range and an increase in heart rate. The appearance of hippocampal theta rhythm preceded the elaboration of conditioned lifting of the paw. The increase of the amplitude of the integrated theta rhythm during the CS significantly differed \((p < 0.05-0.001)\) from that of the background theta rhythm in the same test days.

Along with an increase in amplitude of the integrated theta rhythm, stabilization of its amplitude and an increase of the percentage of theta
activity in the total power of the spectrum was observed during the elaboration of defensive CRs (18).

The amplitude of the integrated theta rhythm in a given trial was related to heart rate, a higher amplitude corresponding to a faster cardiac rhythm (Fig. 2).

![Fig. 2. A, histogram of distribution of heart rates in one of the trials. Ordinate, the number of cases; abscissa, heart rate for 10 sec. B, average amplitude of integrated theta rhythm observed at a given heart rate. Ordinate, amplitude of theta rhythm in relative units; abscissa, heart rate for 10 sec. Dog Zaichik.](image)

To determine the statistical relationship between the change in theta activity and heart rate, the coefficient of correlation was calculated. In background samples, the correlation coefficient was very small ($r = 0.25-0.088$). During the elaboration of defensive CR, the correlation coefficient strongly increased and the relationship between the change of theta rhythm and heart rate became significant.

When the motor CR became automatic, the shifts in hippocampal electrical activity and heart rate decreased appreciably.

Among the objective indices of emotional tension are increased heart and respiratory rates. In these experiments, however, the CR was associated with motor activity which was also accompanied by autonomic changes. This circumstance makes it somewhat more difficult to interpret the changes observed. However, the fact that the heart rate rose upon placing the animal in the experimental situation and increased further during the CS at the stage when the CR was still absent, unequivocally suggests the involvement of an emotional factor at the early stages in the formation of defensive CR. It was during this period of exposure to the CS that the integrated theta rhythm increased in amplitude and stabilized, and the percentage of theta activity increased.
This shows, presumably, that the theta rhythm may be considered as yet another objective index of emotional tension in the animal.

In the process of elaboration of classical alimentary secretory CRs, the overall behavior of the animals changed only slightly over the background. During the CS, the dogs turned to the food dish and salivated. The CS caused a slight increase in frequency and a synchronization of slow wave trains. As a result, the amplitude of the integrated theta rhythm in response to the alimentary CS also increased only slightly, to 106–109% of the baseline level. Statistical treatment of these data showed that increase in amplitude was not statistically significant in most cases. The alimentary CS evoked an increase in the percentage of theta waves in the total power of the spectrum, which was due not so much to an increase in theta rhythm regularity as to a slight decrease of potentials of all other frequencies under study. Similar results have also been reported by Naneishvili and Oniani (14) in weak emotional reactions.

While a positive correlation coefficient was also obtained for the change in amplitude of the integrated theta rhythm and the heart rate change during the formation of alimentary CRs, this correlation was not statistically significant in most trials ($p = 0.2–0.1$).

Thus, during the elaboration of CRs accompanied by very slight increase in emotional tension (as indicated by little autonomic change), the changes in electrical hippocampal activity were also very small and inconsistent.

However, by altering the experimental conditions, greater changes in hippocampal electrical activity and heart rate were obtained in response to the same alimentary CS. Thus, for instance, upon transition from food trials to trials with conditioned switching-over 1 both the heart rate and the amplitude of the integrated theta rhythm increased in response to the trigger signal not only in the defensive part of the experiment but also for trials with food. In these trials, too, the amplitude of the integrated theta rhythm during exposure to the trigger was greater in the defensive than in the alimentary situation (Fig. 3). In both these situations, there was an increased in correlation between the change in amplitude of the integrated theta rhythm and the heart rate.

The above results indicate that during exposure to alimentary and defensive signals the hippocampal electrical activity exhibits unidirect-

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1 In conditioned switching-over experiment the same stimulus (trigger signal) was reinforced by food or shock depending on the situation. The action of fan signalled the nociceptive reinforcement, whereas in the absence of fan food reinforcement was applied. In each situation 4–5 trials were given.
ional changes consisting in an increase of frequency and regularity of slow waves. Thus, while at the background level slow waves at 3.5–4.0 Hz were most characteristic, during the CS there appeared a theta rhythm at 5.0–5.5 Hz. These findings are consistent with those of several authors who also dealt with heterogeneous CRs and who likewise failed to observe noticeable differences between hippocampal electrical activity upon exposure to alimentary and defensive conditional stimuli (7, 10, 11, 24).

Fig. 3. Amplitude of integrated theta rhythm in response to CS in food and defensive situations in three sessions (13, 16, 22). Abscissa, trials; ordinate, amplitude of integrated theta rhythm in arbitrary units. Dog Kutsii.

Despite the unidirectional character of changes in hippocampal electrical activity, the degree of these changes was much greater for defensive than for alimentary CSs. This lends support to the supposition that theta waves are associated with emotional tension. However, it is also necessary to consider the possibility that these differences might be due to different effector responses in the alimentary and defensive CRs, all the more so since many (6, 16, 20, 23) consider the hippocampal theta rhythm only as a correlate of so-called “voluntary” movements. In my experiments too, I have repeatedly observed the appearance of synchronized rhythms in the interstimuli periods preceding the lifting or lowering of the paw.

That the hippocampus is involved in the organization of motor acts is also suggested by the observation that animals with destroyed hippocampus exhibit enhanced motor activity.

However, for the following reasons it is not possible to assign all cases of appearance of the hippocampal theta rhythm merely to the initiation of voluntary movements. As is known, synchronized waves are associated only with goal-directed movements (8, 22). During the elaboration of the defensive CR, a synchronized theta rhythm was observed.
during to the early phase of conditioning prior to the formation of a local, conditioned lifting of the paw. When the motor habit had stabilized and became automatic, and the animals were able reliably to avoid the US, the observed shifts in hippocampal electrical activity became appreciably smaller (7, 18). Some authors have failed to observe any increase in the synchronization of hippocampal theta rhythms during performance of elaborated movements by the animal (24). Increasing the uncertainty of the experimental situation through a transition from an alimentary trials to those with switching-over upon presentation of a trigger stimulus, resulted in an increase of the amplitude of the integrated theta rhythm along with an increase of heart rate in food and defensive situation.

The correlation of the hippocampal response with the overall behavior of the animal is also well seen upon action of the switching signal in experiments with conditioned “switching-over”. The switching on a ventilating fan signalled the transition to a defensive situation, which was accompanied by an increase in heart and respiratory rate, and an enhancement in regularity of the theta rhythm (Fig. 4). Switching off of the fan (Fig. 5) signalled transition from a defensive to an alimentary situation, and decreased the heart and respiratory rates, and caused a short-term synchronization of slow wave trains. The motor response was absent in both cases.

The above findings show that when an emotional tension develops in the animal, the hippocampal theta rhythm appears, even in the absence of a motor response. The differences in the degree of change obser-
ved during the elaboration of alimentary and defensive CRs appear to have been mainly determined by the different interrelative force of the unconditioned reflexes underlying the responses being elaborated and did not depend on the quality of the reinforcement used. This was particularly well seen when comparing the changes arising in hippocampal electrical activity during the elaboration of defensive CRs and of avoidance reactions using aversive stimulation of another dog. These experiments were conducted in the following manner. Two dogs were placed in a chamber attached to different benches 50 cm apart. Shock was applied to the hind limb of the “victim” dog. The stimulation was sufficient to cause not only a local lifting of the paw but also whining and yelping by the dog. In the second, “observer” dog, the left forepaw was fixed to a lever so that by raising the latter it could switch off the shock applied to the “victim” dog.

A conditioned avoidance reflex in response to painful stimulation of the partner was elaborated in three of the six dogs. Upon stimulation of the “victim”, the “observer” dogs exhibited a strong increase in heart rate and a slight increase in the synchronization of slow theta activity (Fig. 6).

The heart rate was increased not only in those dogs which had elaborated a response whereby the partner was relieved of the stimulus, but also in the dog Laskovyi which had not elaborated a motor response despite many trials with passive paw lifting and stimulation of both the dogs with current (Table I). Averaged data have shown that the increase in amplitude of the integrated theta rhythm was very small (Table I).
Fig. 6. Conditioned avoidance response in dog Belka upon nociceptive stimulation of its partner (Tuzik). 1, total power of EEG from amygdala (Channel I) and from hippocampus (Channel II); 2, hippocampal EEG; 3, 4, amygdala EEG; 5, EKG; 6, 7, delta, and theta cycles as isolated by analyser from hippocampal EEG; 8, paw lifting; 9, application of shock to the other dog.

Table I

<table>
<thead>
<tr>
<th>Dog</th>
<th>Heart rate for 10 sec</th>
<th>Increase (%)</th>
<th>Integrated theta rhythm in arbitrary units</th>
<th>Increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Background</td>
<td>Upon painful stimulation of partner</td>
<td>Background</td>
<td>Upon painful stimulation of partner</td>
</tr>
<tr>
<td>Laskovyi</td>
<td>14.8</td>
<td>18.7</td>
<td>26.3</td>
<td>86.9</td>
</tr>
<tr>
<td>Belka</td>
<td>15.4</td>
<td>17.2</td>
<td>11.6</td>
<td>73.6</td>
</tr>
<tr>
<td>Snezhok</td>
<td>13.2</td>
<td>18.7</td>
<td>41</td>
<td>92.7</td>
</tr>
</tbody>
</table>

but was statistically significant in 66–87% of all trials. The changes in amplitude of the integrated theta rhythm were quite variable, not only from one experiment to another but also in the same experiment. The increase in the percentage of theta waves in the total power of the spectrum was less marked than upon action of the defensive CS.

The study of the correlation between the change in heart rate and that in the amplitude of the integrated theta rhythm in those experi-
ments in which one ("observer") dog stayed in the chamber has revealed a low correlation coefficient in most cases. In those experiments in which two dogs were in the chamber, the positive correlation was increased, but the number of trials with a significant positive correlation was low, on the average 21–66\% of the total number. This appears to be due to the fact that even in those trials in which the heart rate and theta rhythm synchronization were strongly marked, these changes were rath-

Fig. 7. Change in hippocampal electrical activity in dog Laskovyi. A, nociceptive stimulation of partner (Tuzik). 1, 2, hippocampal EEG; 3, EKG; 4–6, delta, theta and alpha activity as isolated by analyser from hippocampal EEG; 7, paw lifting; 8, application of shock. B, nociceptive stimulation of dog Laskovyi. I, total power of hippocampal EEG; 2–4, different hippocampal leads; 5, EKG; 6–8, delta, theta and alpha cycles as isolated by analyser from hippocampal EEG.
er short-lasting and were reversed following cessation of stimulation of the partner.

Thus, painful stimulation of the partner caused noticeable increases in heart rate and synchronization in slow wave trains in the “observer” dog. Because these changes were also observed in those trials in which a motor response was absent (Fig. 7A), they appear to be due to an emotional tension. Since the animals performed actions leading to cessation of defensive behavior of the partner, this emotional tension may be referred to as negative. The lack of prolonged after-effects, calm behavior during intertrial intervals, low correlation between the changes in amplitude of the integrated theta rhythm and the heart rate — all suggest a relative weakness of the emotional excitation caused by the defensive behavior of the partner. This is well demonstrated by comparing the records obtained for the same animal when it is stimulated vs. when its partner is stimulated (Fig. 7AB). As can be seen from Fig. 7A, when current was applied to the partner, changes in hippocampal electric activity showed a rather rapid return to the initial level. When, on the other hand, current was applied to the dog itself Laskovyi, a synchronized theta rhythm was recorded throughout the experiment after which the dog refused to enter the chamber for several days and exhibited panting and anxiety.

The above results indicate that during the elaboration of the avoidance CR to painful stimulation of the partner, changes in the hippocampal theta rhythm were, on the average, less marked than during the development of defensive CRs. However, in these experiments, as in those with defensive CRs, the US was negative for the animal and the elaborated instrumental responses had similar effector manifestations. Therefore, the smaller increase in amplitude of the integrated theta rhythm in the experiments using painful stimulation of the partner can be accounted for only by a lesser degree of the excitation underlying the response being elaborated.

DISCUSSION

The present study has shown that both the frequency and regularity of hippocampal theta waves and the heart rate increase during the elaboration of different types of CRs. Changes in hippocampal electrical activity during the formation of instrumental defensive CRs were usually limited to the period of establishment of the new habit and strongly decreased when the response became automatic. In experiments with “switching-over”, the increase in the regularity of hippocampal slow potentials and in the heart rate in response to the trigger stimulus per-
sisted for a longer period of time. These findings make it possible to as-
sociate the occurrence of a hippocampal theta rhythm with an emotion-
al tension in the animal. It was during this period that there also occurred an increase of the positive correlation between the changes in am-
plitude of the integrated theta rhythm and the heart rate. That the hippo-
campal theta rhythm is related to the emotional tension has also been pointed out by Beritov (5), Konorski, Santibañez-H. and Beck (10) and Oniani and Ungiadze (15). According to the information theory of Simonov (21), an emotion is a reflection by the brain of the magnitude of a requirement and of the probability of its satisfaction. As shown by Pigareva (17), the hippocampus belongs to the system of brain struc-
tures which evaluate the probabilistic characteristics of the environment. In this context, it is understandable why synchronized rhythms arise at the early phase of conditioning when the animal does not yet possess enough information for organizing its behavior. From this point of view it may be supposed that the origin of the orienting reflex and the perfor-
ance of non-automatized movements also involve the neural appa-
ratus of emotions, because both the orienting response and non-automat-
tized goal-directed movements are associated with a degree of pragma-
tic uncertainty.

No less important for the degree of emotional tension is, according to Simonov, the magnitude of the requirement underlying the motivat-
ional behavior observed. Thus, as shown by Mekhedova (13), when the requirement for food was increased through food-deprivation, appreci-
ciable increase of emotional tension was observed. In the present ex-
periments, this was manifested in the fact that the degree of emotional tension during the elaboration of alimentary CRs and the avoidance response to nociceptive stimulation of the partner, was smaller than during the development of defensive CRs. This difference appears to have been determined by the difference in the relative force of excitation underlying the reflexes being elaborated.

REFERENCES


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