Hippocampal EEG and Behavior: Changes in Amplitude and Frequency of RSA (Theta Rhythm) Associated With Spontaneous and Learned Movement Patterns in Rats and Cats

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Slow electrical activity was recorded from the dorsal hippocampus in rats during running in a motor-driven wheel, swimming, conditioned avoidance (running, jumping), lever pressing for food and sleep, and in cats during walking in a treadmill, eating and lapping milk. Large-amplitude clear rhythmical slow activity (RSA) was recorded from the hippocampus proper and smaller amplitude RSA and fast activity was recorded from the dentate gyrus-CA 4 area. This was also observed in acute rat preparations. During large movements (run, jump) RSA amplitude was up to 6 times greater than during small movements (head turn, lever press). Initiation of movement was associated with an increase in RSA frequency, which was most pronounced with forceful movements. During steady running or swimming in the rat frequencies of 7-9 Hz were recorded and during prolonged running up to 8 hr RSA was unchanged. RSA frequency did not vary with the speed of steady running or steady locomotor patterns of swimming, walking, trotting or galloping. RSA frequency and amplitude may reflect different mechanisms; frequency was normal after hippocampal electrographic seizures, amplitude was depressed; frequency varied directly with core temperature (27-42°C) but amplitude remained constant. Increases in RSA frequency and amplitude during paradoxical sleep were associated with muscular twitches, suggesting that forebrain motor mechanisms were activated. The results are interpreted in support of the idea that RSA is related to higher level control of voluntary movement.

Several investigators have reported that hippocampal EEG activity in cats is changed as a result of training in a learning task, but there has been

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disagreement as to the exact nature of the change and its functional significance. According to Grastyán, Lissák, Madaras and Donhoffer (1959) the initial response to a novel stimulus is a “desynchronization” of hippocampal activity. As learning proceeds, the initially novel stimulus becomes an effective conditioned stimulus and rhythmical slow activity (RSA) appears associated with prominent orienting movements. When the learned response is well established, orienting movements disappear. RSA is no longer elicited by the conditioned stimulus and the hippocampal response to it is again one of “desynchronization.”

These findings have seemed contradictory to reports by Adey and others that hippocampal activity consists largely of RSA during first exposure to novel stimuli and that RSA does not disappear even after prolonged over-training in both approach and avoidance situations. However, Adey and his colleagues have shown that a well-established approach response is accompanied by RSA of a higher frequency than is seen in the same animal early in training. Also, in a choice situation, correct responses are associated with higher frequency RSA than incorrect responses (Adey, 1962; Adey, 1967; Pickenhain and Klingberg, 1967). Changes in the phase relations of RSA waves to the CS or to RSA waves at other sites have also been reported.

Some recent findings on the relation of hippocampal EEG to behavior suggest an explanation of these divergent results. Hippocampal RSA occurs during what may be called voluntary behavior (including walking, running, jumping, rearing, swimming, digging, manipulation of objects with the fore-limbs, isolated movements of the head or one limb, and shifts of posture) but is generally absent during more automatic behavior (including licking, biting, chewing, chattering the teeth, vocalization, shivering, facewashing, scratching, pelvic thrusting, defecating, urinating, ejaculating, and piloerection). If behaviors from both groups occur simultaneously RSA is always present. The details of RSA wave morphology are related to the details of the performance of the accompanying voluntary movement. For example, large movements, involving most of the body, are accompanied by RSA of larger amplitude and (usually) higher frequency than slight movements. Consistent EEG-behavior relations are observed only if the recording electrodes are placed in relation to the pyramidal cells in such a way that clear large-amplitude RSA can be recorded. Electrodes placed in the dentate area or subiculum yield a great deal of fast activity which can obscure RSA, particularly when its amplitude is low (Bland and Vanderwolf, 1972a; Sainsbury, 1970; Vanderwolf, 1969; Vanderwolf, 1971; Vanderwolf, Bland and Whishaw, 1972; Whishaw, 1972).

In the experiments described here a more detailed analysis was made of the changes in frequency and amplitude of RSA during learned as well as spontaneous movements in rats and, in addition, the observations were extended to cats. The results suggest that the changes which occur during training can be understood in terms of changes in behavior per se.
SUBJECTS

The subjects were 51 male hooded rats weighing 250-350 g at the start of the experiments. Four adult cats (1 male, 3 female) were also used.

APPARATUS AND PROCEDURE

Under pentobarbital anaesthesia, stainless-steel electrode pairs were implanted in the dorsal hippocampal formation and in the neocortex according to standard stereotaxic procedures. Two to four pairs of bipolar electrodes were implanted at various locations in the rats and six pairs of electrodes were implanted in the cats. An electrode pair consisted of two 250 μm wires insulated to within 0.5-1.0 mm of the tips. One wire tip was 1.0 mm longer than the other and the two tips were spread about 1 mm apart. This type of stagger-tip electrode was used because it produces large-amplitude activity, virtually free of artifacts, when placed with one tip above the hippocampal pyramidal cell layer and one below it (Vanderwolf, 1971; Vanderwolf, Bland and Whishaw, 1972).

After a 2-wk recovery period hippocampal activity was recorded continuously on a Grass Model VII ink-writing polygraph during a series of behavioral tests including:

(1) Wheel running. Ten rats were gradually adapted to running in a 183-cm circumference motor-driven wheel at a speed of 33 ft/min. They were then given a single continuous 8-hr session of running. Two cats were given similar adaptation and testing in a 46 × 115-cm treadmill in which a canvas floor was driven at a speed of 56 ft/min. The two remaining cats were given shorter periods of walking. Two of the rats continued running 6-9 hr/day until they had run a total of 100 hr. Five remaining rats were trained to run at speeds of 33, 60, 90 and 144 ft/min.

(2) Bar pressing. The ten rats trained to run in the wheel were also trained to press a lever in an apparatus consisting of an elevated platform measuring 56 × 38 cm, and equipped with a lever and control equipment which would deliver 45-mg food pellets to a food cup located 5 cm away from the lever. Each bar press was recorded automatically on the polygraph chart and, in addition, a capacitance-sensing device (Electrocraft of Canada) similar to the unit described by Griffiths, Chapman and Campbell (1967) was placed beneath the food cup and connected to the polygraph so that the movement of an animal retrieving a food pellet was also automatically recorded on the chart. Initially, the animals were placed on a 22-hr wet mash, food-deprivation schedule and were then placed on the stand for 15 min daily for 10-15 days. Five of the animals received bar-press training prior to wheel running, for the other five the order was reversed.
(3) Avoidance tests. In different experiments, 16 rats were tested in a one-way test and a jump-avoidance test. The one-way avoidance apparatus was a two-compartment box 25 × 45 cm high, with a black "start" compartment 20 cm long and a white runway 60 cm long. The grid floor could be electrified by a Harvard inductorium powered by a 1.5-V dry-cell battery. A door hinged on the side of the apparatus separated the start compartment from the runway. The jump-avoidance apparatus was a plywood box (30.5 × 30.5 × 30.5 cm) mounted on rubber blocks. Rats were taught to jump a distance of 11 in to a shelf running around the outside edge of this box in order to avoid electric shock from the inductorium. The movement sensor, placed beneath a metal plate fixed to the side of the box, gave an almost instantaneous indication of the time of the initiation of jumping. The accuracy of the device was checked by recording the change in resistance between adjacent bars of the grid floor as the rat broke contact with them. By means of a telescoping device placed within the avoidance box, the ledge could be raised so that the rats could also be taught to jump a distance of 22 in. In the avoidance tests the animals were given 20 test trials 1 day following 4 days of preliminary training (20 trials/day). When jumping heights were compared, the same 4-day preliminary training on each height was followed by 5 days of testing at each height.

(4) Swimming. Seven rats were taught to swim a distance of 120 cm in a straight alley 30 cm wide filled with 38 °C water. The rats were first given 20 training trials a day for 4 days and were then given one 20-trial test session.

In addition to these more formal tests, electrical activity was recorded from 14 rats and 4 cats during a number of spontaneous behaviors including feeding, drinking, slow wave and paradoxical sleep.

The amplitude and frequency of hippocampal electrical activity in eight rats was differentially manipulated by producing electrographic seizures in the hippocampus and by changing core temperature. Electrographic seizures were produced by stimulation of the hippocampus unilaterally through a standard recording electrode with 6-12 V, 60-Hz, 0.1-msec duration rectangular pulses from a Grass S-4 stimulator and SIU-5 stimulation isolation unit. Changes in electrical activity resulting from stimulation were recorded from the contralateral hippocampus. In experiments in which core temperature was changed, rats had their fur clipped and were cooled in 10 °C water or heated under a heat lamp in the manner described by Whishaw and Vanderwolf (1971). Rectal temperature was monitored during temperature change with a telethermometer (Yellow Springs Instrument Co.) through a probe inserted to depth of 6.5 cm.

In acute preparations ten rats were anesthetized with urethane and placed in a stereotaxic instrument. Concentric recording electrodes with a diameter of 300 μm with a tip which protruded 1 mm were used for recording hippocampal EEG. One electrode was lowered 6 cm from the skull surface in
one hippocampus and a second control recording electrode was lowered 3.25 cm from the skull surface in the other hippocampus. A bipolar stimulating electrode with tips cut straight across was lowered into the lateral hypothalamus. The preparation was allowed 30 min to recover from electrographic seizures and then recording began. The deep recording electrode was raised in 0.25-mm steps and samples of hippocampal electrical activity were taken during lateral hypothalamic stimulation (4-6 V, 100-Hz, 0.1-msec rectangular pulses). The electrode was then lowered to the level producing optimal hippocampal RSA and the location was marked with a small lesion.

In all experiments, the polygraph records were analyzed by inspection and counting. Wave-by-wave measurements of RSA period and amplitude (peak-to-peak) were made using a transparent plastic ruler. Quantitative data were analyzed by analysis of variance procedures (Winer, 1962). At the completion of the experiments the animals were sacrificed under pentobarbital anesthesia and their brains removed. Coronal brain sections, 40 μm thick, were cut using the frozen technique, mounted and stained with thionin.

RESULTS

As shown in previous work in the dorsal hippocampus (see Introduction), clear RSA could be recorded from the pyramidal cell layer, as would be expected from the finding that RSA is generated by these cells (Green, Maxwell, Schindler and Stumpf, 1960). The results obtained from recording in freely moving rats were also confirmed in the acute preparations in which samples were taken from different levels of the hippocampus proper and dentate gyrus (Fig. 1). When the lower tip of the bipolar electrode was in the apical dendrite layer of the pyramidal cells of CA 1-2, large-amplitude clear RSA was recorded. At this time the two tips of the recording electrode lay on either side of the pyramidal cells. More dorsal placements yielded RSA of smaller amplitude while more ventral placements yielded first fast activity mixed with RSA and then only fast activity (mainly 15-50 Hz) when the tip of the lower pole was at the level of the granular cells of the dentate gyrus. The RSA which was produced by hypothalamic stimulation was accompanied by increases in respiration, stretching of the body and some movement of the limbs. Control recordings from the stationary electrode in the contralateral hippocampus showed large-amplitude RSA throughout the period of sampling during lateral hypothalamic stimulation. Histological examination of placements from rats used in the behavioral experiments confirmed this relation between types of hippocampal EEG activity and anatomical placement of the electrode. In all, 29 rats and 2 cats had at least one electrode which produced clear RSA. The remaining placements produced mixed or fast activity. Two placements in the medial thalamus and one in the lateral hypothalamus produced mixed RSA and fast activity. RSA in these placements was related
Fig. 1. The relation between hippocampal EEG activity and electrode placement in an acute urethane-anesthetized rat preparation. The recording electrode was raised in 0.25-mm steps and samples of EEG activity were taken at each locus during stimulation of a lateral hypothalamic placement which produced RSA. C is the control recording taken throughout the tests from the contralateral hippocampus. Note: when the lower tip of the electrode was in the apical dendrite layer of the pyramidal cells (4-5) large-amplitude RSA was recorded. More dorsal placements yield RSA of smaller amplitude while more ventral placements yield first fast activity mixed with RSA and then only fast
to behavior in a way similar to hippocampal RSA. Thirty-one placements in the neocortex (both anterior and posterior) produced fast desynchronized activity during voluntary movements.

Previous reports on the relation between hippocampal RSA and behavior were also confirmed. In the experiments with rats it was found that RSA was present in the pyramidal cell layer in all situations at all times when the animals walked, ran, jumped, swam, pressed a lever, moved their heads, manipulated a piece of food, etc. (voluntary movement), but that RSA was absent if the animals chewed food, washed their faces, vocalized, etc. (automatic movement). If the two types of movements occurred simultaneously (e.g., head movement while chewing) RSA appeared. A similar relation was found in cats. Hippocampal RSA was recorded when cats walked, made orienting movements or moved their heads, but was absent during lapping milk and chewing cat food (Fig. 2). RSA in cats was about 2-2.5 Hz slower than in rats. Other obvious differences in cats were that more fast activity was mixed with RSA in cats than rats and RSA could occur in cats during fixed staring (such as when the cat observes the movement of the polygraph paper, or movement outside the window) although the cats did not make large overt body movements.

EEG with wheel running and treadmill walking. After a week of gradual adaptation to the wheel, ten rats were forced to run for 8 hr at a speed of
33 ft/min. For two of these rats, forced exercise was continued for 6-9 hr/day until they had run a total of 100 hr. Amplitudes and frequencies of RSA accompanying wheel running on the 8-hr test are shown in Fig. 3. There was a slight decline in RSA frequency ($F = 2.85$, $df = 10/90$, $p < 0.05$) which was correlated with the increased number of “rests” (i.e., periods of immobility while the wheel moved the rats passively) taken by the animals as testing progressed, but there was no significant change in the amplitude of RSA. Two cats were given a similar 8-hr walking test in the treadmill and data on RSA are also shown in Fig. 3. RSA was continuously present during walking throughout the entire session, although RSA frequency was about 2-2.5 Hz slower than in rats. In the two rats given forced exercise over a number of days up to 100 hr, RSA was also always present with unchanged frequency and amplitude throughout testing.

**EEG changes with bar pressing.** When the rats were initially placed on the barpress apparatus, large-amplitude RSA was recorded as the animals explored (walking, rearing) the apparatus, however, as bar pressing was established the amplitude of RSA declined. Measurements of RSA from a session of bar pressing compared to wheel running showed a mean decline of 44% of RSA amplitude (range 35-52% in different rats). Examples of the differences in EEG accompanying wheel running and bar pressing are shown for three rats in Fig. 4. In addition to changes in amplitude, it was found that placements which yielded large-amplitude clear RSA during wheel running still
Fig. 4. Electrical activity in the hippocampal formation during bar pressing, eating, and wheel running in three rats. Rat 106. Electrode tips on either side of hippocampal pyramidal cell layer. Note: (1) Large-amplitude RSA during wheel running and breaks in rhythm when rat pauses momentarily, (2) small amplitude RSA during bar-press response, (3) irregular activity during chewing. Rat 119. Electrode tips on border between hippocampus and dentate gyrus. Note: (1) Large-amplitude RSA (more fast activity than in rat 106) during wheel running, (2) less clear-cut RSA during bar pressing. Rat 126. Electrode tips in dentate gyrus. Note: (1) Poorly developed RSA during wheel running, (2) absence of RSA during bar pressing. (3) presence of considerable large-amplitude fast activity at all times. Calibration: 1 sec and 100 μV; half-amplitude filters 1 and 35 Hz.

showed clear RSA during bar pressing (top), while placements which yielded mixtures of fast activity and RSA showed little or no RSA during bar pressing (middle). Placements which showed a great deal of fast activity with RSA during wheel running, showed no RSA during bar pressing (bottom). In rats with multiple implants in the hippocampus clear RSA of reduced amplitude could be obtained from one electrode during bar pressing, while in electrodes which produced faster activity, RSA could be recorded during wheel running, but not bar pressing.
Similar changes in EEG activity were observed during spontaneous behavior (Fig. 5). In six rats with multiple implants it was observed that when
rats made large movements such as walking and rearing large amplitude RSA could be recorded. However, if the animals made smaller movements such as handling a food pellet, or isolated movements of the head, RSA of reduced amplitude was still recorded in sites producing clear RSA, while in sites which produced faster activity mixed with RSA, RSA was inconspicuous or absent.

Changes in RSA frequency with movement initiation. A summary of the changes in RSA frequency with the initiation of movement is shown in Fig. 6.

Fig. 6. 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 (4) 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. Wave number indicates 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 at each point associated with each response for each rat on each of 20 trials.
On the jump-avoidance task the highest frequencies of RSA were concurrent with the jump, with lower frequencies preceding and following the jump \((F = 18.40, df = 20/120, p < 0.001)\). The fastest wave occurred after the rat had left the grid, but before it reached the top of the box. Initiation of running in the oneway avoidance task was also associated with faster RSA frequencies than occurred prior to or during running \((F = 10.40, df = 20/120, p < 0.001)\). Initiation of running in the motor-driven wheel \((60 \text{ ft/min})\) following a “rest” was also associated with higher frequencies of RSA than steady wheel running, during which RSA frequency was constant. In this situation the results were less exact than in the others because the moment of initiation of running had to be judged by eye. However, the results in Fig. 6 show that the expected frequency shift occurred in the RSA record. In the swimming test an increase of RSA frequency with initiation of swimming was not obtained since the rats were struggling when placed in the water and the frequency was already quite high. An analysis of RSA frequency change during steady swimming indicated that there was no significant frequency shift \((F = 0.97, df = 20/120, p > 0.05)\). The movement pattern in rats during swimming is different from running, the rats tuck their front feet up under their chin and paddle with deep thrusting movements of the back feet. The actual extent of the frequency shift in the first three tests is not comparable since the movement detector quite accurately reflects the point of movement initiation in the jump-avoidance task but not in the oneway avoidance task. In the wheel-running task the point of movement initiation was defined as the first RSA wave following the desynchronization of the hippocampal RSA associated with the pause in running. In these tests the analysis was based on 20 trials from each rat in a single test session. RSA frequency changes with different running speeds and jumping heights. An analysis of RSA frequency from five rats trained to run in the motor-driven wheel at speeds of 33, 60 and 90 ft/min indicated that RSA frequency did not change with running speed \((F = 3.81, df = 2/8, p > 0.05\) based on measurement of 16,803 waves or about 2 min at each speed for each rat). At the different speeds different locomotor patterns occurred. The rats walked at 33 ft/min, trotted at 60 ft/min and moved at a fast trot at 90 ft/min. Most rats would not run at higher speeds, but one rat galloped at 144 ft/min. The hippocampal RSA at this speed was not different than during fast trotting at 90 ft/min. These results are summarized in Fig. 7.

When rats were trained to jump 11 and 22 in. out of a box to avoid footshock it was found that the frequency of RSA associated with the higher jump was significantly faster by about 2 Hz than the frequency of RSA associated with the lower jump \((F = 42.88, df = 1/6, p < 0.001)\). The results shown in Fig. 7 were based on analysis of 50 trials for each rat at each height. Since it has been shown that RSA frequency is affected by core temperature (Whishaw and Vanderwolf, 1971) core temperatures were taken before and after jumping for a second group of four rats. If the rats were left on the
Fig. 7. Frequency of RSA in five rats running at three speeds in the motor-driven wheel and four rats jumping 11 in. (solid lines) and 22 in. (dotted lines). The rats walked at 33 ft/min, trotted at 60 ft/min, and moved at a fast trot at 90 ft/min. Each point for each rat represents the mean frequency of a 2-min sample of RSA during steady running. For jumping, the “0” is the wave preceding jump initiation as indicated by the movement-sensing device. At both heights the fastest wave occurred while the rat was in the air but “lift-off” time was 50-100 msec longer on the high jump. The same rats were used for both low and high jumps and each curve is based on 50 trials (10 trials/day) for each rat at each height.

Changes in RSA during paradoxical sleep. Observations of hippocampal EEG were made during sleep for 11 rats. During paradoxical sleep periods of twitching (paws, ears and vibrissae) were recorded by the movement sensor and by observation. Frequency and amplitude measurements of RSA indicated that significantly faster frequencies and higher amplitudes of RSA were obtained during periods of movement than during periods of non-movement. Table 1 shows the mean frequencies of RSA during periods of movement and during periods of non-movement for five rats which were also used in the swimming, bar-press and wheel-running tests. It can be seen from Table 1 that the overall frequency of RSA during paradoxical sleep is lower than that obtained during the waking behaviors. However, RSA frequency from periods of twitching during paradoxical sleep is not significantly different from RSA frequencies obtained during wheel running and swimming in the waking state. The difference is accounted for by the fact that low-frequency RSA, 6 Hz, is present during paradoxical sleep when the rat is not twitching. Frequencies of
TABLE 1
Relation Between RSA Frequency and Behavior

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Mean RSA Frequency</th>
<th>RSA Frequency of individual rats</th>
<th>Total No. of periods measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Wheel running (60 ft/min)</td>
<td>7.7</td>
<td>7.1 7.6 7.8 7.9 8.3</td>
<td>5,519</td>
</tr>
<tr>
<td>(2) Paradoxical sleep (twitching)</td>
<td>7.6</td>
<td>7.6 7.4 7.5 7.7 7.7 7.9</td>
<td>6,536</td>
</tr>
<tr>
<td>(3) Swimming</td>
<td>7.6</td>
<td>7.4 7.4 7.6 7.6 7.9</td>
<td>2,450</td>
</tr>
<tr>
<td>(4) Bar pressing</td>
<td>7.2</td>
<td>7.2 7.2 7.2 7.2 7.4</td>
<td>2,513</td>
</tr>
<tr>
<td>(5) Paradoxical sleep (overall)</td>
<td>6.7</td>
<td>6.5 6.7 6.8 6.8 6.8</td>
<td>9,088</td>
</tr>
</tbody>
</table>

\[a\] The mean RSA frequencies for behaviors 1, 2 and 3 were not significantly different \((p > 0.05)\).

\[b\] The mean RSA frequencies for behaviors 1, 2 and 3 were significantly higher than those for behaviors 4 and 5.

\[c\] The mean frequency for behavior 4 was significantly higher than that for behavior 5.

RSA during bar pressing are lower than during swimming, wheel running, or twitching during paradoxical sleep. Many movements made during paradoxical sleep are quite brief and often these are not accompanied by an increase in RSA frequency and amplitude. At other times movements are vigorous and could last from 5-15 sec. Measurements of wave periods of only these very vigorous movements gave very high mean frequencies (mean of 8.3 Hz) in five rats.

The relation of RSA amplitude to movement. The results of the examination of RSA amplitude during a number of behaviors in four rats is shown in Fig. 8 and an example of amplitude differences in one rat is shown in Fig. 9. To obtain samples of head and body movements and food handling the animals were maintained in close proximity to the polygraph in an observation box. After 4 days, when the animals had adapted to the laboratory environment, samples of EEG activity were taken during: (1) slight postural changes (such as rolling slightly to one side when resting), (2) head movements in the absence of associated body or limb movements, (3) movements of the front paws during handling a food pellet, (4) jumping, (5) walking, (6) struggling when held, (7) swimming and (8) paradoxical sleep. Large movements (jumping, walking, swimming, struggling) were associated with large RSA amplitude while smaller movements of the head or paws or body were associated with smaller RSA amplitudes. During paradoxical sleep a wide range of amplitude of RSA was recorded but during periods of movement amplitude was larger than during periods of nonmovement (Fig. 9). It can also be seen from Fig. 8 that RSA amplitude varies as a function of both electrode locus and behavior.
HIPPOCAMPAL EEG

Fig. 8. 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 a movement-sensing device) were measured.

Dissociation of RSA frequency and amplitude. Hippocampal RSA frequency has been found to be directly proportional to core temperature (Weiss, 1963; Whishaw and Vanderwolf, 1971) but, while frequency can change 5-fold between 24 and 42°C, RSA amplitude remains relatively unchanged. Electrical stimulation of the hippocampus results in suppressed activity during stimulation followed by large-amplitude after-discharges which continue for 10-60 sec, following which the record is depressed but gradually recovers amplitude over 15-30 min (Petsche, Stumpf, Gogolak, 1962; Whishaw and Deatherage, 1971). Hippocampal fits were produced in four rats as they ran in the motor-driven wheel at a speed of 60 ft/min. RSA amplitude was reduced by 50-75% following the fit, but frequency was unchanged. Fits were also induced in four rats at normal core temperature, 27 and 42°C. Examples of the results obtained with one rat are shown in Fig. 10. The amplitude of RSA following the fit was depressed but frequency was unchanged. Frequency changed with core temperature, however, amplitude remained constant. Thus, the two variables of RSA could be manipulated independently. Details of some aspects of behavioral changes which accompany these changes in hippocampal RSA activity have been reported elsewhere (Whishaw and Deatherage, 1971; Whishaw and Vanderwolf, 1971).
Fig. 9. Electrical activity at a single hippocampal site during sleep and various behaviors. Note: RSA during paradoxical sleep, jumping, struggling when held in the hand, swimming and head movement; large-amplitude irregular activity during sitting still while alert and while chattering the teeth; and the irregular slow activity and “spindling” during slow-wave sleep and small-amplitude irregular activity 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 movements; swimming, jumping 11 in. and jumping 22 in. Calibration: 1 sec, 500 µV; half-amplitude filters, 0.3 and 75 Hz. Electrode placement: CA 1, hippocampus major.

DISCUSSION

As shown in previous work on the dorsal hippocampus (Vanderwolf, 1969; Vanderwolf, Bland and Whishaw, 1972) clear RSA can be recorded from the pyramidal cell layer, as would be expected from the finding that RSA is generated by these cells (Green, Maxwell, Schindler and Stumpf, 1960). The largest amplitudes and the clearest records were obtained from
Fig. 10. The effect of changed core temperature and electrically induced hippocampal electrographic seizures on hippocampal RSA. Frequency of RSA varied with core temperature while RSA amplitude remained unchanged. Following a hippocampal electrically induced electrographic seizure amplitude was reduced in the postictal stage but frequency was related to temperature just as in the normal state. Calibration: 1 sec, 500 µV, half-amplitude filters 0.3 and 75 Hz. Electrode placement: CA 1 hippocampus major.

bipolar electrodes placed with one tip just above the pyramidal cell layer and one just below it. Placements in the dentate gyrus yielded fast activity, often with a large amplitude with varying admixtures of RSA. Previous reports on the relation between hippocampal RSA and behavior were also confirmed. In the experiments with rats it was found that RSA was present in the pyramidal cell layer in all situations at all times when the animals walked, ran, jumped, etc. (voluntary movement), but that RSA was absent if the animals chewed food, washed their faces etc. (automatic movement). The results also indicated that the mere repetition of the same motor act does not lead to a disappearance of hippocampal RSA, when such activity was initially present. RSA persisted in rats trained to run for 8 hr in a motor-driven wheel and in cats trained to walk for 8 hr in a treadmill. RSA was also present when rats...
ran or jumped to avoid shock and when rats swam in a water alley. These experiments confirm previous findings that RSA persists despite long-continued training (Adey, 1962; Adey, 1967; Adey, Dunlop and Hendrix, 1960; Bremner, 1964; Elazar and Adey, 1967; Pickenhain and Klingberg, 1967; Vanderwolf, 1969).

Quite different results were obtained from electrode placements which did not yield clear RSA. Placements in or close to the dentate gyrus area yielded fast activity, often of a large amplitude and with varying admixtures of RSA. At such sites it was observed that RSA was present on the first day of training in the bar press task but that such activity disappeared when the rats had learned to press the bar regularly. This result confirms the earlier observations of Grastyán et al. (1959).

Two main factors appear to determine whether or not RSA disappears during training. One is anatomical; the other behavioral. First, as already stated, an electrode pair placed near Ammon's pyramids (preferably with the tips straddling the cell layer) is likely to yield clear large-amplitude RSA, while an electrode in the dentate gyrus yields mainly fast activity (15-50 Hz). At many sites mixtures of the two types of activity are obtained. Second, there is a relation between the amplitude of hippocampal RSA and the extent of muscular field activated during the associated voluntary movement. The RSA associated with head movement, a lever-press response, or with the manipulation of an object, or a small body movement during resting, has a smaller amplitude than the RSA associated with walking, struggling, running, swimming and jumping. If a recording electrode is so placed that it yields a mixture of RSA and large-amplitude fast activity, the RSA associated with a small movement may be obscured, although the RSA associated with an extensive movement appears clearly. Thus, early in training in the bar-press task a great deal of walking and rearing (exploration or orienting responses) occurs and RSA is prominent. As training proceeds, particularly in a task like bar pressing, extensive movements tend to disappear and RSA becomes reduced in amplitude but is still clearly identifiable at some electrode placements, but in other electrode loci which produce more fast activity it disappears.

Such “disappearance” of RSA is not peculiar to learning test situations and may be observed very frequently during spontaneous behavior. During slight movements in a rat small-amplitude RSA could be recorded from one hippocampal site while activity at another site might be described as desynchronized. During walking and rearing, however, recognizable RSA appeared at both sites. Presumably RSA was present in both hippocampi during the smaller movements but is obscured in some placements by faster activity. In conclusion, training in rats can produce a disappearance of RSA from some sites in the hippocampus but this effect is due to relations between hippocampal activity and the details of motor activity and is not directly related to the activity of the learning process.
Observations made in four cats suggest that behavior and hippocampal activity are related in much the same way in these animals as in rats, even though the cats were not trained in more formal learning tasks. RSA occurred consistently during walking, climbing, head movements but was often absent during immobility, eating, lapping milk, washing the face or licking the fur. As in rats, RSA was continuously present in cats for periods of up to 8 hr as the animals walked steadily in a motor-driven wheel. The major difference is that the frequency of RSA in the cat is about 2.5 Hz lower in the cat than in the rat. This observation would seem to confirm the earlier suggestion of species differences in RSA in “higher” animals (Green and Arduini, 1954). In addition, more fast activity was observed in even the best RSA placements in cats than was found in rats. This may confirm earlier suggestions of species differences in this regard (Green and Arduini, 1954) or simply indicate that it is more difficult to make optimal electrode placements in this species. RSA could also occur during fixed staring in cats, such as when an animal stares at a moving object without making large overt body movements. Brown (1968) has reported that RSA activity at this time is slower than during periods of movement. It has previously been suggested that such slower RSA activity which occurs during periods of non-movement represents a central state of preparation for movement (Harper, 1971; Vanderwolf, 1969; Whishaw, 1972). When a cat “orients,” the movement involves muscles of the neck and trunk as well as the limbs. The accompanying RSA often has an amplitude even larger than RSA associated with walking. This may explain why RSA has so often appeared to be related specifically to orienting behavior. If the relations of hippocampal EEG and behavior in the present study apply equally to cats in more formal learning tasks, then the records of Grastyán et al. (1959) and Bennett and Gottfried (1970) which contain considerable fast activity and have a relatively low amplitude (50-200 μV), may not have been optimal in showing the smaller RSA amplitude which occurs with slight movements in learning tasks as well as during spontaneous behaviors. At any rate, many instances of hippocampal desynchronization reported by Grastyán et al. (1959) occur when cats were aroused by a novel stimulus but did not move. Records taken at other times and defined as desynchronized clearly contain smaller amplitude RSA mixed with fast activity (e.g., see Fig. 5C, p. 415, Grastyán et al. 1959).

Other reports of changes in hippocampal activity during learning (Ady, 1962; Elazar and Adey, 1967) suggest that there is a relation between frequency of RSA and learning. Changes in frequency have been reported in several studies with changes in movement pattern (Kamp, Lopes da Silva and Storm van Leeuwen, 1971; Lopes da Silva and Kamp, 1969; Pickenhain and Klingberg, 1967; Vanderwolf, 1969). These results were confirmed in the present study. In addition, it was found that RSA frequency in rats was constant over time during steady movements of swimming and running and that frequency did not vary during running at different speeds. The frequency
of RSA also did not appear to be related to the pattern of steady movement since the same RSA frequency was recorded during walking, slow trotting, fast trotting, galloping and swimming. RSA frequency did increase during the initiation of movement of running and jumping. In the case of running, frequency dropped to a lower level once movement was initiated. When RSA frequency was compared for rats jumping different heights, higher frequencies of RSA were recorded with the higher jumps than with the lower jumps. These results suggest that changes in frequency may be related to the force or acceleration with which movement is initiated. Lower frequencies of RSA are associated with head movements and paw movements during food handling (Vanderwolf, 1969) and during bar pressing for food than during swimming and running. Lower frequencies with these movements may be due to the slower initiation and termination of these movements, which might be related to levels of excitation in brainstem systems which activate RSA and movement (Klemm, 1970).

In a learning experiment when an animal is trained to approach food in an alley, response latency declines progressively. This means that the animal is likely to pause or stand still while moving its head, shifting posture, etc., early in training and will walk promptly toward the goal later on. Therefore, it is expected that mean RSA frequency will rise during training in a task of this type. Similarly, it is well known that response latency is longer on “incorrect” trials than on “correct” trials on a discrimination task, a fact that leads to widespread use of response latency as a measure of learning. Since longer latencies mean more head movements, more shifts in posture etc., mean RSA frequency will be lower on “incorrect” than on “correct” trials. Elazar and Adey (1967) report such a result, but also state that the frequency spectrum was wider on incorrect than correct trials. Presumably this means that the animals engaged in several behaviors on incorrect trials but walked steadily on correct trials. Unfortunately, detailed observations on behavior were not provided.

It has been reported that RSA can be recorded from the neocortex at times when hippocampal RSA occurs (Parmeggiani, 1967; Yamaguchi, Yoshii, Miyamoto and Itoigawa, 1967). Probably this represents a physical spread of current from a source in the hippocampus since no phase reversal of the waves is obtained in the cortex, but one is present in the hippocampus (Green, Maxwell, Schindler and Stumpf, 1960). Landfield, McGaugh and Tusa (1972) have reported that the amount of cortical RSA following a one-trial learning test may be related to the amount of learning. Landfield et al. (1972), however, did not attempt to distinguish between rhythmical waves of hippocampal origin and other rhythmical waveforms, such as “spindles” which can also be recorded in the neocortex (Pickenhain and Klingberg, 1965). Whishaw and Vanderwolf (1971) reported that spontaneous cortical spindles (rhythmical waves with a frequency of 6-9 Hz) occur during immobility or “freezing.” Thus, such waveforms would be expected in rats that perform well
in a passive avoidance of the type used by Landfield et al. (1972). Further, the idea that hippocampal RSA is related to learning or memory is contradicted by many reports that RSA is not present during learned immobility responses (Bland and Vanderwolf, 1972b; Dalton and Black, 1968; Paxinos and Bindra, 1970).

One condition in which long periods of RSA occur unaccompanied by a large amount of skeletal activity is during paradoxical sleep. Frequency and amplitude measurements of RSA from records obtained from rats indicated that significantly higher frequencies and larger amplitudes were obtained during periods of movement than periods of non-movement. Reports on paradoxical sleep in other species including the cat, dog and rabbit also indicate an increase in amplitude and frequency during periods of movement (Harper, 1971; Okuma and Haruo, 1966; Parmeggiani and Zanocco, 1963; Yoshii, Shimokochi, Miyamoto and Ito, 1966). Kiyono, Kawamoto, Sakakura and Iwamura (1965) have reported an overall increase in RSA frequency during “makeup” paradoxical sleep (i.e., the increased paradoxical sleep which follows deprivation of 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.” Pompiano (1967) has presented much evidence that high levels of activity occur in cerebrospinal (motor) pathways during paradoxical sleep but that movements are prevented by a tonic postsynaptic inhibition of spinal motor neurons throughout paradoxical sleep and by a phasic presynaptic inhibition during the movement periods of paradoxical sleep. Twitches of the extremities are apparently caused by excitation “breaking” through the inhibition. The observation of Jouvet (1967) that selective suppression of descending inhibition with lesions to the brainstem results in cats which awaken and display “hallucinatory” behavior on entering paradoxical sleep also supports this interpretation. The significance of central activation of motor systems during paradoxical sleep is not known, however, the results suggest that RSA may be related to activation of movement mechanisms during sleep in much the same way as it is during waking.

The results of the present study suggest that RSA amplitude is related to the extent (i.e., possibly the total number of motor units activated together) of muscular activation while frequency shifts are related to the acceleration with which movement is initiated. However, frequency is also increased during large as compared to small movements and the present data do not show clearly the degree to which RSA frequency and amplitude are independently related to behavior. It is not clear why RSA frequency is constant during steady movements of different patterns and speeds, but a similar effect has been reported for running at different speeds in response to brainstem electrical stimulation (Bland and Vanderwolf, 1972b). Possibly, such a system allows forebrain circuits greater sensitivity in monitoring and guiding changes in movement pattern. The suggestion that amplitude and frequency are partly independent is consistent with a previous report that the wave
period and amplitude of RSA are not clearly related (Vanderwolf, 1969). The demonstration in the present study that amplitude and frequency can be manipulated independently following electrographic seizures and by manipulating core temperature also lends some support to this idea and may lead to further measures which will aid in clarifying the role of these parameters in the higher level control of movement.

The experiments described here show that during movement RSA may (a) remain unchanged, (b) change in frequency, (c) change in amplitude, or (d) disappear, depending on the recording site and the behavior engaged in by the animals. The conclusion that these changes are not directly related to learning may seem extreme. It might be argued that a more refined analysis of hippocampal activity (averaging, cross- or auto-correlation or unit activity) might reveal changes related to learning. However, refined measures of cerebral activity cannot solve the problem alone. They must be matched by equally refined measures of behavior, such as simultaneous EMG recording from large numbers of muscles. In experiments performed to see whether hippocampal changes correlate better with presumed occurrence of learning or recall than with the performance of particular types of movement, it was found that when the learned response consisted of immobility (Dalton and Black, 1968; Paxinos and Bindra, 1970) or of licking water (Young and Black, 1970) or of squealing (Vanderwolf, Bland and Whishaw, 1971) RSA did not occur consistently but was uniformly present when the learned behavior consists of running, jumping, swimming, bar pressing, etc. Therefore, it seems unlikely that RSA is directly connected with learning, memory, or recall as such, independent of the type of behavior which is performed.

REFERENCES


