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Activity of Hippocampal Formation Neurons in the Monkey Related to a Conditional Spatial Response Task

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SUMMARY AND CONCLUSIONS

To analyze neurophysiologically the functions of the primate hippocampus, the activity of 905 single hippocampal formation neurons was analyzed in two rhesus monkeys performing a conditional spatial response task known to be impaired in monkeys and in man by damage to the hippocampus or fornix. In the task, the monkey learned to make one spatial response, touching a screen three times when he saw one visual stimulus on the video monitor, and a different spatial response, of withdrawing his hand from the screen, when a different visual stimulus was shown. Fourteen percent of the neurons fired differentially to one or the other of the stimulus-spatial response associations. The mean latency of these differential responses was 154 ± 44 (SD) ms. The firing of these neurons was shown to reflect a combination of the particular stimulus and the particular response associated by learning in the stimulus-response association task and could not be accounted for by the motor requirements of the task, nor wholly the stimulus aspects of the task, as demonstrated by testing their firing in related visual discrimination tasks. Responsive neurons were found throughout the hippocampal formation, but were particularly concentrated in the subicular complex and the CA3 subfield. These results show that single hippocampal neurons respond to combinations of the visual stimuli and the spatial responses with which they must become associated in conditional spatial response tasks and are consistent with the suggestion that part of the mechanism of this learning involves associations between visual stimuli and spatial responses learned by single hippocampal neurons.

INTRODUCTION

Bilateral damage to the temporal lobe in humans can cause anterograde amnesia (16, 30, 32, 33). A number of structures are damaged, including the hippocampus and the amygdala. Experimental investigations have been performed to determine which structures are crucial in producing the amnesia and to analyze the neural bases of the different types of amnesia (8, 34). In the monkey it has been shown that the ability to remember a list of objects in a recognition task is impaired by combined damage to the hippocampus and amygdala, but is much less affected by damage to the hippocampus or amygdala separately (17). In analyses of the way in which the hippocampus could contribute to a memory deficit, it has been shown that tasks which are particularly affected by hippocampal damage in the primate include tasks in which both an object and the place in which it was seen must be remembered (8, 10, 20). It is thus of interest that *H.M.*, and humans with right temporal lobe damage, were impaired in remembering the positions in which objects had been placed on a board (31). We have recently reported that 9.3% of neurons in the hippocampal formation respond to the spatial position of stimuli presented in a multiple object-place task (22).

Another task in which monkeys with hippocampal lesions are impaired is a conditional spatial response task in which one spatial response must be made to one stimulus, and a different spatial response to another stimulus (9, 29). In this task, the monkey had to press a panel three times to obtain a reward when one visual stimulus was shown. When a different stimulus was shown, the monkey had to withhold pressing the panel for 3 s to obtain reward. On each day, the monkey had to learn the correct responses to a new pair of stimuli. It was found that monkeys with damage to the hippocampal system showed slower rates of learning this task than control monkeys (29). It is important that this task cannot be solved by reward association learning, as each of the responses is equally associated with reward. Instead, the task requires the monkey to learn which spatial response to make to one visual stimulus to get the reward and which different spatial response to make to a different stimulus also to obtain the reward. It is also important that this conditional spatial response task requires new stimulus-motor response associations to be learned rapidly and flexibly [in the study by Rupniak and Gaffan (29) new associations were required every day], and so is different from habit learning, in which relatively fixed motor responses are learned gradually (18). It is of interest in relation to the study of mechanisms of human memory that humans with right temporal lobe damage also have deficits in learning conditional spatial response tasks (21).

To analyze the functions of the hippocampus in the primate and to advance the understanding of amnesia, we are recording the activity of single hippocampal neurons in the monkey during the performance of tasks known to be affected by hippocampal damage and in related tasks. In this paper we describe the activity of single neurons in the conditional response task described above. It was of interest to determine whether neuronal activity was related to the performance of the task and if so, whether the neuronal activity was related to the stimulus processing required for the task, to the motor responses being made in the task, or to

the associations required to perform the task. The findings indicate that a considerable proportion of hippocampal neurons show differential responses in the task and that the activity of the majority is not unconditionally related to the stimuli used or to the movements made. The results provide evidence that their activity is related to forming associations between visual stimuli and spatial responses and that the activity of single hippocampal neurons reflects these associations, and not just the visual stimuli or the motor responses. The results are thus consistent with the hypothesis (24) that the rapid learning within the hippocampus of such associations is one of the functions it performs which enables it to contribute to the type of learning investigated here.

METHODS

Conditional spatial response task

The task may be described as a conditional response task with symmetrical reinforcement, nonspatial stimuli, and arm movement responses. The response requirement was for three arm reach responses when one stimulus was shown [fixed ratio three (FR3)] and for response omission [differential reinforcement of omission (DRO)] when the other stimulus was shown. The task is therefore abbreviated to FR-DRO. The task was designed to be very similar to that used by Rupniak and Gaffan (29), so that neuronal responses recorded might be related to the deficit in the performance of the task shown by Gaffan and Harrison (9) to be produced by damage to the hippocampus.

The monkey initiated each trial by pressing a central key placed 25 cm in front of him. This sounded a 0.5-s signal tone (400 Hz) to enable him to fixate the video monitor placed 30 cm in front of him, just above the response keys. At the end of the tone, a discriminative visual stimulus appeared on the screen. To one of the stimuli (FR stimulus), the monkey had to press a response key (situated 3 cm to the right of the observing response key) three times within 3 s to obtain reward. In later experiments a similar procedure was used except that the FR response required the monkey to touch the monitor screen. These trials thus required a fixed ratio (FR3) type of response, which had to be completed within 3 s. The reward was fruit juice which could be obtained by licking a tube in front of the mouth, and its availability was indicated on the video monitor by a white circle presented for 1 s. If the monkey failed to touch 3 times within 3 s, the first touch after this period resulted in a white square appearing on the video monitor, which indicated that reward would not be obtained by licking. If the monkey licked, he obtained aversive saline solution. To the other stimulus of a pair (DRO stimulus), the monkey had to withhold pressing the response key for 3 s. If he performed this response correctly, the circle appeared on the screen, and he could lick to obtain fruit juice reward. This was thus a differential reinforcement of omission (DRO) contingency. If he pressed the response key incorrectly, then the square appeared at the end of the 3-s period, he obtained saline if he licked, and the next trial was delayed by an extra 5 s beyond the normal intertrial interval of 5 s. A PDP11 computer sequenced the trials randomly. The task was completely computer-controlled to ensure that no influence by the experimenters on the monkey's behavior or on the neuronal activity was possible. The computer switched the stimuli on and off for each trial and synchronized its data collection so that the stimulus was turned on at the start of the 21st bin of a peristimulus time histogram. The stimuli were displayed 30 cm from the monkey on a color video monitor which subtended 12° at the retina. The stimuli were pictures digitized from the television. The resolution of these images was 256 pixels wide by 256 pixels high. The stimuli were digitized by the computer and loaded into an AED512 video framestore from the computer disk for each trial.

The task could be run with different pairs of stimuli as the discriminative stimuli, but once a pair had been chosen, that pair was used for at least 50 trials. Measurement of the activity of the neuron when the monkey performed the same task but with a different pair of stimuli was one way in which evidence was obtained on whether the responses of hippocampal neurons were related simply to movements made by the monkeys. Further evidence on this, and on whether the neuronal responses were related simply to the particular stimuli being shown, was obtained when reversals of the task were required, that is when the monkey was asked to learn to make the opposite responses to the two stimuli.

Visual discrimination task with an arm movement response

To obtain further evidence on whether any neuronal activity recorded in the above task might be related to the movements made in the task, the following control task was also performed by the monkeys. This was a Go/NoGo visual discrimination task with asymmetrical reinforcement using the same arm reaching movement response made to the same location as that used in the task described above. It is abbreviated to OA (operant task with arm movement response). The monkey initiated each trial by pressing the central key placed 25 cm in front of him. This sounded a 0.5-s tone to enable him to fixate the video monitor placed 30 cm in front of him, just above the response keys. At the end of the tone, a discriminative visual stimulus appeared on the screen. To one of the stimuli (the "Go" stimulus, usually a white circle presented for 1 s, and usually different from the discriminative stimulus used in the FR-DRO task described above), the monkey could press the response key (situated 3 cm to the right of the observing response key) to obtain a delivery of the fruit juice reward into his mouth. As described above, a similar version of the task was used in later experiments with a touch-sensitive screen. (In both these versions of the OA task, the arm movement was made to the same position in space as in the conditional response task, to show whether neuronal activity was related simply to arm movements.) To the other of the stimuli (the "NoGo" stimulus, usually a white square, and usually different from that used in the FR-DRO task described above), the monkey had to withhold pressing the response key. If the monkey pressed the key, he obtained aversive saline solution. No reward was given on NoGo trials, so that the task could be solved by stimulus-reinforcement associations, in that one stimulus was always associated with reward and the other with saline.

Conditional left-right spatial response task

This task was also a conditional response task with symmetrical reinforcement using nonspatial stimuli, but required the monkey to make a spatial arm movement response to the right or the left side. Following one of the stimuli a reach response to touch the right side of the monitor was required, and to the other stimulus a reach to touch the left side was required. The stimuli consisted of black and white sine-wave gratings of different spatial frequencies and orientations and were not spatially restricted to any position on the screen. This is thus a simple example of a task in which one spatial response (go right) must be learned to one stimulus, and a different spatial response (go left) must be learned to another visual stimulus.

Other tasks

The monkeys were also trained to perform a visual discrimination task with a lick response, which was run as a further control to ensure that any results obtained were not due to obtaining

reinforcement, licking for fruit juice, etc. This task is abbreviated to OP (operant task). If a circle, the positive discriminative stimulus (S+), appeared on the monitor, the monkeys could lick to obtain a fruit juice reward, and if a square of the same area and luminance, the negative discriminative stimulus (S−), appeared the monkey had to withhold licking in order to avoid aversive hypertonic saline. A 0.5-s tone preceded the presentation of the stimulus, and if the monkey was fixating correctly before the stimulus appeared, he had sufficient time to perform the discrimination and obtain multiple licks of the fruit juice tube in the short (1.0 s) period in which the stimulus was on. This procedure was designed to ensure fixation of the stimuli (28), and was also used in the OA memory task. The order of presentation of the stimuli was randomized. The electrooculogram (EOG) recordings confirmed that this procedure resulted in consistent fixation of the video monitor screen on which the stimuli were presented.

Recording techniques

The activity of single neurons was recorded with glass-insulated tungsten microelectrodes [after Merrill and Ainsworth (15), but without the platinum plating] in two rhesus macaque monkeys (*Macaca mulatta*) (weight 3.0–4.5 kg) seated in a primate chair using techniques that have been described previously (25). The monkeys had been implanted under thiopental sodium anesthesia with stainless steel holders on which an adaptor could be fitted for the later daily recording sessions. The action potentials of single cells were amplified using techniques described previously (28), were converted into digital pulses using the trigger circuit of an oscilloscope, and were analyzed on-line using a PDP11 computer. The computer collected peristimulus rastergrams of neuronal activity for each trial and displayed, printed, and stored each trial, as well as computing the peristimulus time histogram by summing trials of a given type. To facilitate latency measurements, the cumulative sum distribution was calculated from the sum peristimulus time histogram. For each trial the number of action potentials occurring in a 500-ms period (and a 250-ms period) starting 100 ms after the stimulus onset was printed. This period was chosen because the neurons studied responded to visual stimuli with latencies which were typically $\geq$100 ms, and the monkeys consistently fixated the stimuli for >500 ms. Fixation of the stimuli was confirmed using permanently implanted silver/silver chloride electrodes for EOG recording. The EOG recordings provided eye position with an accuracy of 1–2° and were sampled by the computer every 10 ms and saved with the action potentials for each trial.

X-radiographs were used to locate the position of the microelectrode on each recording track relative to permanently implanted reference electrodes and bony landmarks such as the posterior tip of the sphenoid bone (1). The position of cells was reconstructed from the X-ray coordinates taken together with serial 50-μm histological sections stained with cresyl violet which showed the reference electrodes and microlesions made at the end of some of the microelectrode tracks.

Treatment of results

For each cell, measures of responses were calculated from the total number of action potentials occurring on each trial during periods starting 100 ms after the presentation of the discriminative stimuli and lasting for 250 or 500 ms. A Student's t test was performed to determine whether the neuron responded differently on the two types of trials in a task. The level of significance required was taken as 0.05, although for the majority of differentially responding cells, the actual level of significance achieved was $P < 0.001$. A few such significant results might be expected by chance among the population of neurons from which recordings were made. To test whether more cells had significant results than would be expected by chance, the number of cells with significant results at each level (e.g., $P < 0.05$, $P < 0.01$, and $P < 0.001$) found using the t test was compared using a χ^2 test with the number that would be predicted by chance.

The latency of neuronal responses or the differential latency of the neuronal response, that is the latency at which it fired significantly differently for the different trial types, was determined using cumulative sum and running mean statistics. The cumulative sum (36) was calculated on-line, using 18 prestimulus bins as the reference. The point at which the slope of the cusum changed was taken as the latency. Running mean t tests, which compared the mean number of neuronal spikes in 18 prestimulus bins with the mean number of spikes in 2, 3, 4, or 5 poststimulus bins, were performed over the sums of trials of any one condition, over the difference of the sums of trials of two conditions, or over the cumulative sums of these arrays of values, to provide further confirmation of the latency at which each neuron responded differently on the two types of trials.

RESULTS

The activity of 905 neurons was recorded during the performance of the FR-DRO conditional response task in two monkeys. Four hundred fifty-nine neurons altered their firing rate in the task. Of these, 28 responded as the monkey initiated a new trial, 189 responded during the cue period or at the time of image onset, 5 neurons were activated when the trial was aborted (see above in METHODS), and 192 neurons responded differently in the two types of trials. It was found that 63 of these 192 neurons with differential responses had activity which was unconditionally related to the movements that the monkey was required to make (e.g., reaching), as shown by the neuronal responses found in either the OA task (in which 111 of the 192 neurons were tested) or with alternative stimulus pairs but the same movement responses, in the FR-DRO task (see below). These neurons were therefore classified as movement-related and were not studied further. The remaining 129 neurons with differential responses in the task (14% of the total sample) thus had activity which was not movement-related, yet which was differential in the conditional response task. There were many more such neurons with statistically highly significant response as indicated by the t tests than would be predicted by chance (as tested by a χ^2 test, $\chi^2 = 1014$, df = 2, $P \ll 0.001$). This extremely highly significant χ^2 test result shows that the population of neurons did not respond differently in the different conditions of the conditional response task just by chance. The responses of these 129 neurons form the main subject of this paper. The responses of the neurons which were unresponsive in this study are described elsewhere (4, 26).

An example of the activity of a single hippocampal neuron in the conditional response task is shown in the top part of Fig. 1. This neuron fired more on DRO than on FR trials. Rastergrams and peristimulus time histograms are shown. The latency at which the neuron responded significantly differently on DRO and FR trials was 120 ms, as shown by cusum tests and by running mean statistics. The latencies of the monkey's touch responses to the key, as shown by each T, were ~300–400 ms. The lower part of the figure shows that the neuron did not fire during the performance of the visual discrimination task which used the same arm reach response (OA trials), so that its activity was not movement-related.

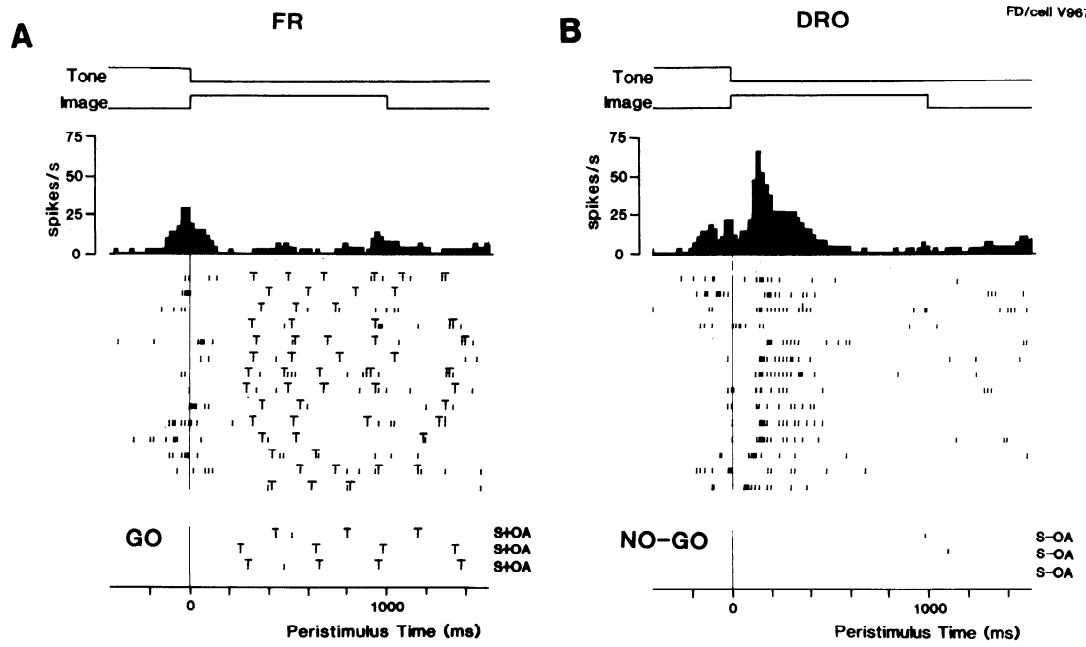

FIG. 1. Example of the activity of a neuron in the conditional response task. Peristimulus rastergrams and time histograms show that the neuron responded on DRO trials with a latency of 120 ms. The visual stimulus appeared at *time 0*, when a 0.5-s warning tone ended. On FR trials the neuron did not show the poststimulus firing evident on the DRO trials. Each touch made by the monkey is shown by a T. Responses of the neuron were not related to arm movements being made in the conditional response task, for in a simple visual discrimination task (OA) shown at the bottom of the diagram, the neuron did not respond either when the monkey made the arm movement response when a reward-related visual stimulus was shown (S+ OA or GO trials), or when the monkey withheld the arm movement response in order to avoid obtaining saline when the S− visual stimulus was shown (S− OA or NO-GO trials). The operant task with an arm movement response (OA) was also tested on this neuron to determine if its activity was unconditionally related to the motor aspect of these tasks. This neuron was recorded in the dentate gyrus.

An example of the firing of another single hippocampal neuron in the conditional response task is shown in the top part of Fig. 2. This neuron fired more on FR than on DRO trials. Rastergrams and peristimulus time histograms are shown. The latency at which the neuron responded significantly differently on FR and DRO trials was 100 ms, as shown by cusum tests and by running mean statistics. The latencies of the monkey's touch responses to the key were ~300–400 ms. The middle part of the figure shows that the neuron did not fire during the performance of visual discrimination tasks which used the lick response (OP trials) with the same visual stimulus pair, showing that the response of the neuron could not be accounted for simply by a response to the stimulus used in the FR-DRO task. Figure 2 (middle) also shows that the neuronal response was not related unconditionally to the movement made in the FR-DRO task, in that the neuron did not respond when the monkey made the same arm movement to touch the screen in the visual discrimination task (OA trials). Thus the neuron responded to the combination of the particular stimuli and the particular responses which had been associated by learning in the conditional response task.

The responses of another neuron tested with different stimulus pairs in the conditional response task are shown in Fig. 3. With stimulus pair 1, the neuron increased its firing on FR trials with a latency of ~200 ms and decreased its firing on the DRO trials with a latency of ~100 ms. When the monkey performed the conditional response task using two different stimuli, as shown on the right of the diagram under "stimulus pair two," the neuron decreased its firing on the FR trials with a latency of ~100 ms and did not respond on the DRO trials. This set of results shows that the neuron did not have responses that were unconditionally related to the movements being made, which were the same with stimulus pair 1 and stimulus pair 2. In other tests (see below), it was shown that such neurons did not respond unconditionally to the stimuli (e.g., when the stimuli were shown in different tasks), so that such neurons respond in conditional response tasks to combinations of particular stimuli with particular responses.

Of the 129 neurons which responded differentially in the conditional response (FR-DRO) task, 60 responded on the FR trials (43 with an increase of activity, 17 with a decrease), and 51 on the DRO trials (39 with an increase, 12 with a decrease). Eighteen neurons showed a combination of an increase and a decrease in the different task conditions during the period over which activity was analyzed. Those neurons showing an increase of firing had a mean spontaneous firing rate of 16.2 spikes/s and had a mean response firing rate of 34.2 spikes/s. Neurons which displayed a decreased response to one of the stimuli had a mean spontaneous firing rate of 26 spikes/s with a mean response rate of 10.1 spikes/s. Finally those neurons showing a combination of an increase to one stimulus but a decrease to the other had a mean spontaneous firing rate of 27.7 spikes/s and a mean response increase to 39.4 and decrease to 17.2 spikes/s.

The differential response latencies of these neurons after the onset of the discriminative visual stimuli are shown in Fig. 4. The mean latency was 154 ± 44 (SD) ms for neurons

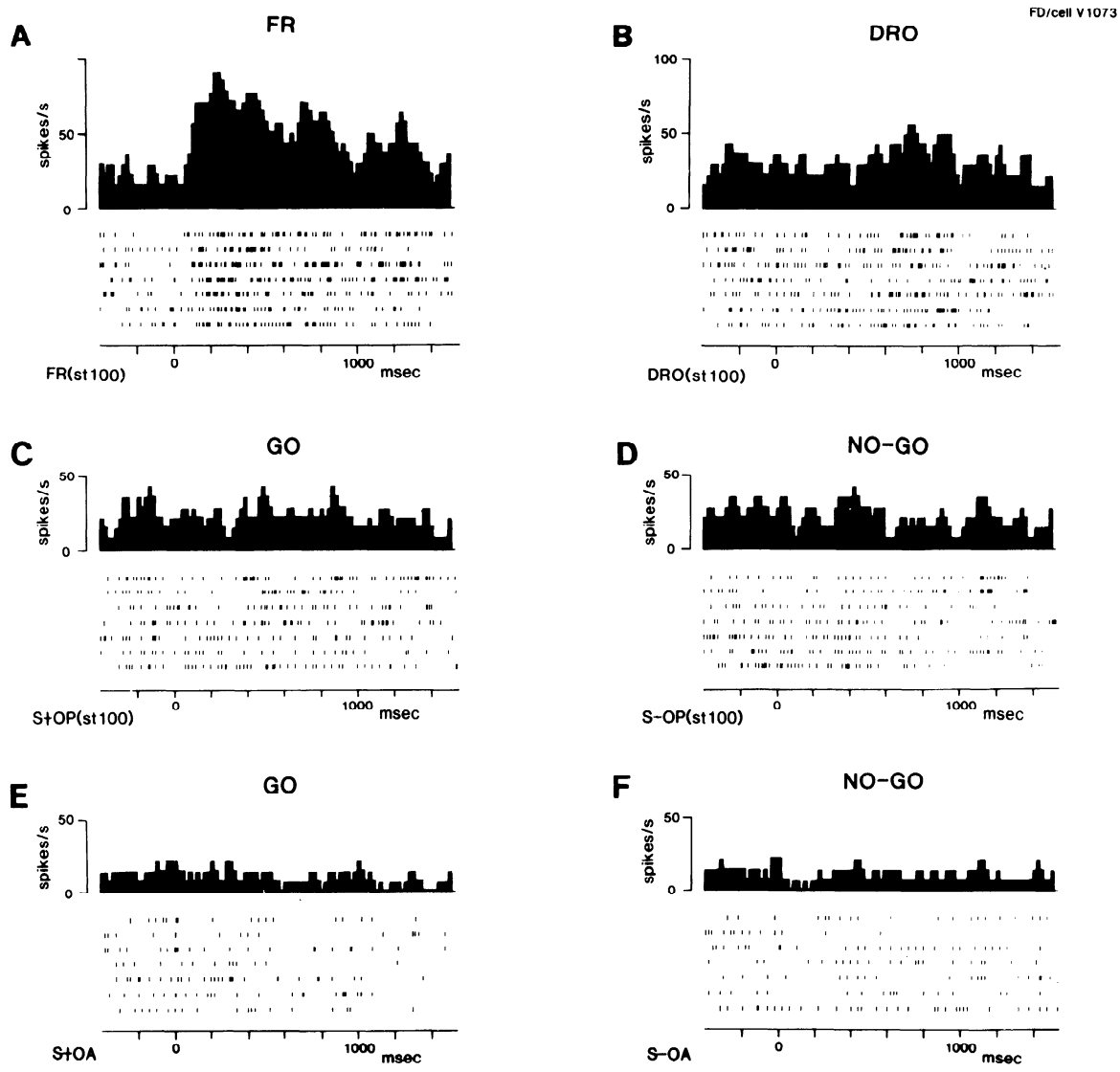

FIG. 2. Example of the activity of another neuron in the conditional response task that responded more on FR than on DRO trials (*A* and *B*). The conventions are as in Fig. 1. The latency for the differential responses was 100 ms with respect to stimulus onset. No differential activity was found in a visual discrimination task using the same stimulus pair as in the FR-DRO task, so that the neuronal responses in the conditional response task were not simply due to the stimuli shown (*C* and *D*). In this visual discrimination task, on S+ OP trials the monkey made licks to the FR stimulus to obtain fruit juice reward and on S− OP trials did not lick to the same stimulus used on DRO trials in order to avoid obtaining saline. *E* and *F* show that in the OA task, the neuron did not respond on S+ OA trials, even though the same arm movement was being made as in the FR trials. Thus the neuronal response in the FR-DRO (conditional response) task was not unconditionally related to movement. This neuron was recorded in the CA3 region of the hippocampus.

belonging to the main peak whose latency was ≤260 ms. The motor response latencies of the first touch made on FR trials were typically in the range of 300–400 ms. The electromyogram associated with the responses might be expected to start ~100 ms before the behavioral response was detected (cf. Ref. 24), so that the response latencies of most of the hippocampal neurons probably preceded the movements made by the monkeys in the task.

The nature of the responses in the task is next considered by assessing whether they responded in the other tasks.

Of the 129 neurons with differential responses in the conditional response (FR-DRO) task 85 neurons were also recorded from in the OA task, in which the same arm movement response was made, to investigate whether the differential neuronal responses were simply related to arm movements. (In the OA task, arm movement responses to one stimulus were always rewarded and if made to the S− stimulus were associated with the delivery of aversive saline, so that this is a Go/NoGo task, not a conditional response task.) Examples of recordings made in this control OA task are shown on at the bottom of Figs. 1 and 2. Fifty-three of the 85 neurons (62%) did not respond in the OA task, whereas 30 neurons (35%) responded differentially but in the opposite direction to that observed in the FR-DRO task (see Table 1). Thus the responses of these neurons in the FR-DRO task were not unconditionally related to the movements made in the task, and so they were not classified as movement-related. Of the 85 neurons, 2 (2.3%) responded in the same way in the OA task as in the FR-DRO task, but neither of these two

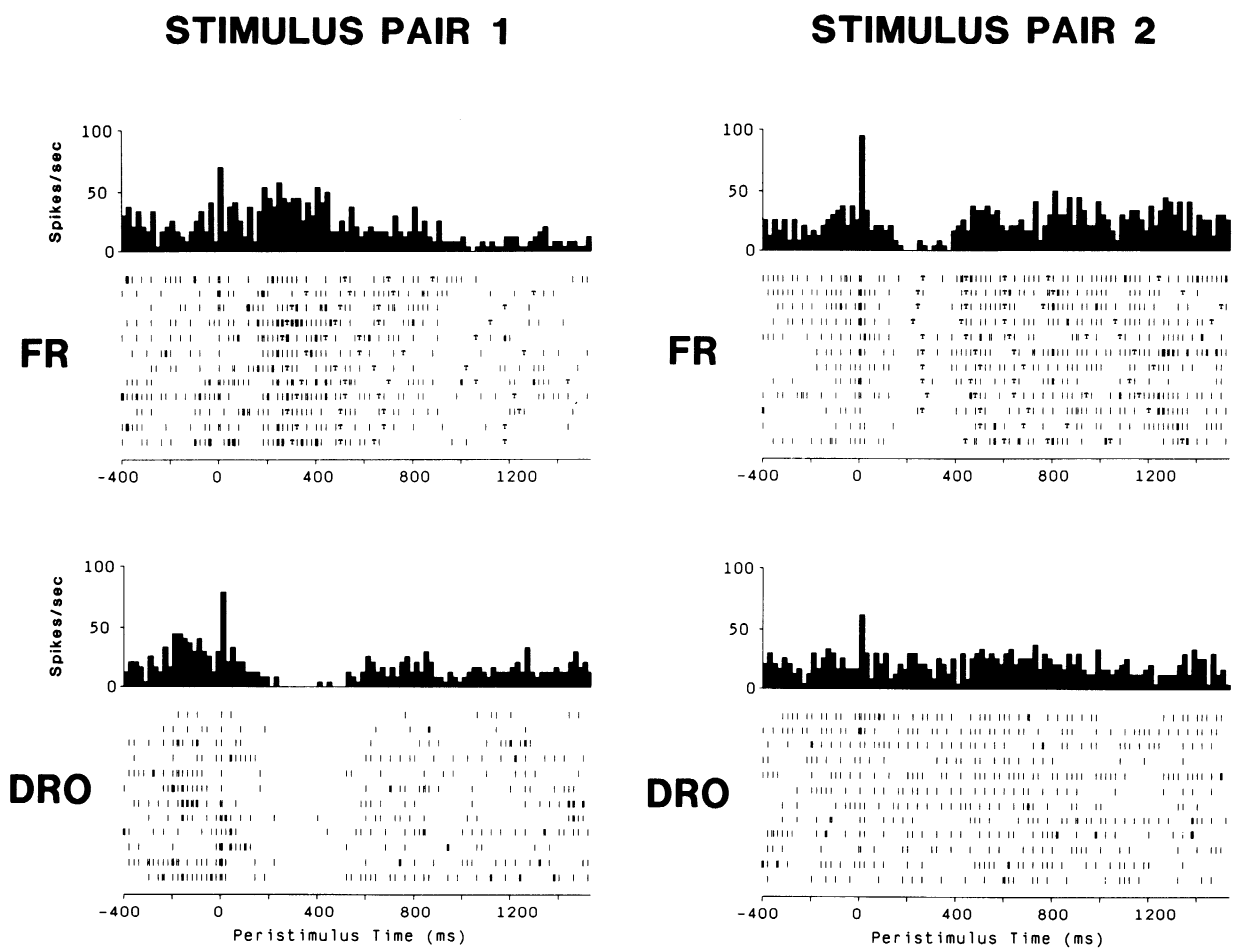

FIG. 3. Responses of another hippocampal neuron which was tested with different stimulus pairs in the conditional response task. The conventions are as in Fig. 1. Stimulus pair 1: the neuron increased its firing on FR trials with a latency of ~200 ms and decreased its firing on the DRO trials with a latency of ~100 ms. Stimulus pair 2: when the monkey performed the conditional response task using two different stimuli, the neuron decreased its firing on the FR trials with a latency of ~100 ms and did not respond on the DRO trials. This set of results shows that the neuron did not have responses which were unconditionally related to the movements being made, which were the same with stimulus pair 1 and 2.

neurons responded consistently using the other FR-DRO pair (see e.g., Fig. 3) of stimuli, so that none of these 129 neurons with differential responses had movement-related activity.

The possibility that some of these neurons were coding whether or not a motor response should be initiated (in this case a lick movement) was tested by analyzing the activity of 43 of the 129 differential neurons in the visual discrimination task which used a lick response. In this (OP) task the monkey had to lick to receive reward when one visual stimulus was shown, but to make no response when the other visual stimulus was shown (otherwise saline was obtained). Of the 43 neurons tested 29 showed no response, and 13 showed a response which was inconsistent with that shown in the FR-DRO task (i.e., the neuron responded to NoGo if it had responded to FR in the FR-DRO task or to

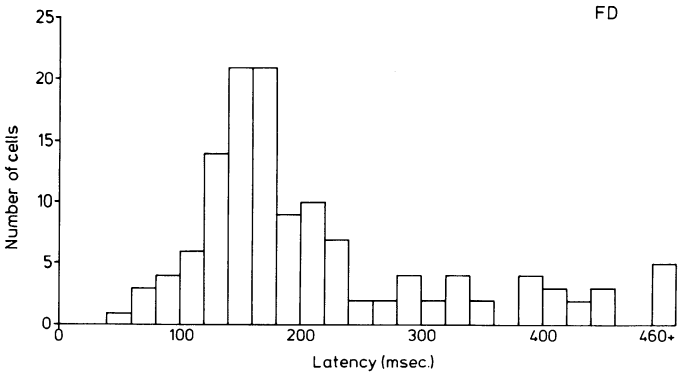

FIG. 4. Frequency histogram of the differential response latencies of the neurons recorded in the FR-DRO task in the 2 monkeys. The main peak occurred between 80 and 260 ms. The mean latency, for latencies <260 ms, was 154 ± 44 (SD) ms.

TABLE 1. *Nature of the activity of neurons with differential responses in the FR-DRO task when tested in other tasks*

	No Response	Different Response	Similar Response	Total
OA	53	30	2	85
OP	29	13	1	43
Alternative FR-DRO pairs	19	26	14	59

Values stated are no. of neurons. FR-DRO, fixed ratio-differential reinforcement omission; OA, visual discrimination task with arm movement response; OP, visual discrimination task with lick response; alternative FR-DRO pairs, different pairs of visual stimuli substituted into the FR-DRO task.

Go if it had responded to DRO) (see Table 1). (Thirty-one of these 43 neurons were tested with the OA task.) Only one neuron showed a response which was consistent with a hypothesis of movement initiation accounting for the responses in both tasks, but even this neuron did not fire simply in relation to movement initiation, in that it responded on the opposite type of trial when an alternative pair of stimuli was used in the FR-DRO task.

The second possibility considered is that the neurons' responses were related to the particular stimulus shown in the conditional response (FR-DRO) task. One type of evidence on this came from comparing the neuronal responses when the monkey was working with different stimulus pairs to indicate FR as opposed to DRO (as already mentioned above). Of 59 neurons for which evidence is available, 14 (24%) responded similarly in the task (e.g., on FR trials) even when different stimulus pairs were used (see Table 1). Thus the responses of these neurons were not stimulus specific. It may be noted that these neurons were not just movement related either, in that all 14 tested failed to respond in the OA task. Thus their responses are neither unconditionally stimulus-related nor unconditionally response-related. Of the 59 neurons tested with different stimulus pairs in the FR-DRO task, 26 (44%) responded differently when different stimulus pairs were used. For one of these neurons, it was possible to show using a reversal of the FR-DRO stimuli that the responses occurred to one of the stimuli independently of whether it signified FR or DRO, and this neuron thus had a stimulus-related response. The responses of the remaining 25 neurons could have been related either to the stimulus shown or to the combination of the stimulus and the motor response required to that stimulus. Some additional data on whether these neurons were unconditionally stimulus-related comes from the nine neurons with comparable responses (e.g., on Go trials) in the FR-DRO and OA tasks. In that these neurons had differential responses in these two tasks in which different stimuli were used, the neurons did not have stimulus-specific responses for one of the stimuli used in the tasks. Further evidence that the majority of these neurons did not have responses which were unconditionally stimulus-related is that during the learning of this FR-DRO task, the responses of 16 out of 24 neurons tested altered when the monkey learned the responses to make to the stimuli, so that these neuronal responses could not be accounted for by stimulus-evoked activity alone (6). Further evidence consistent with this conclusion is that 3 out of 3 neurons tested with the same stimuli in the FR-DRO task and another task (e.g., OP or OA) did not show the same responses to the same stimuli in the different tasks. These findings together show that the responses of at least the majority of the neurons with differential responses in the FR-DRO task could not be accounted for simply by unconditional stimulus-related activity.

It was of interest that of the neurons tested with alternative pairs of stimuli in the FR-DRO task only 19 (32%)

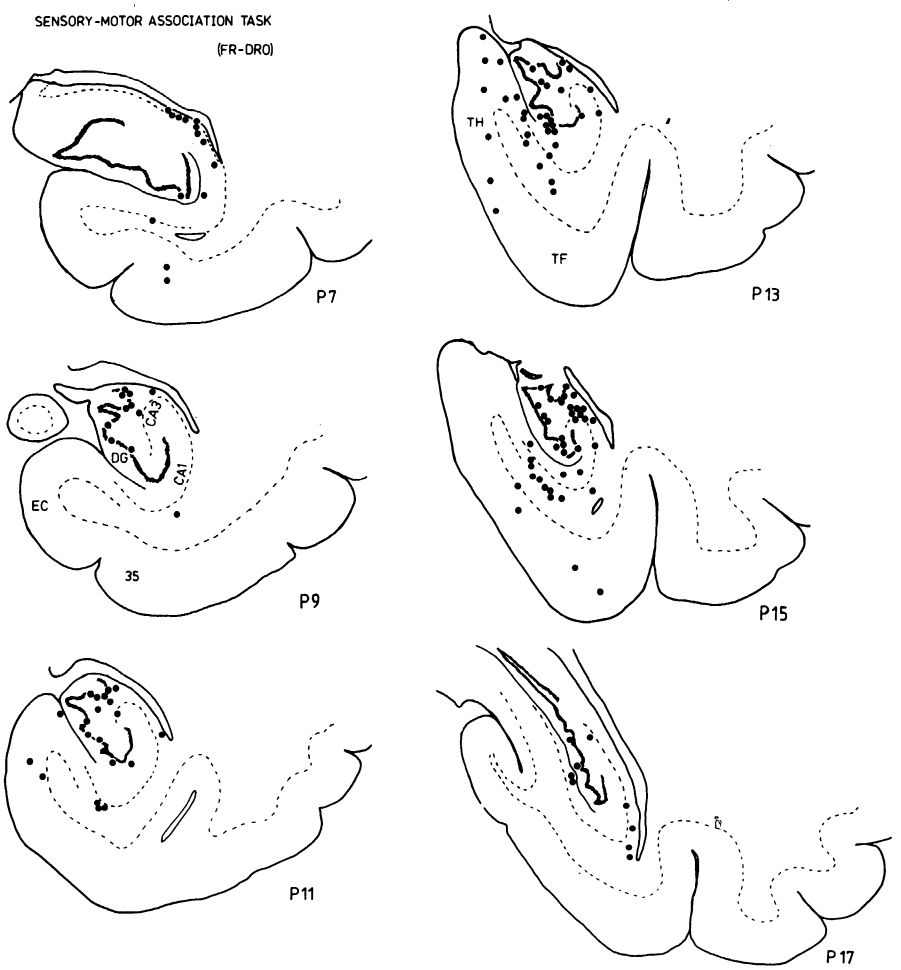

FIG. 5. Recording sites at which differentially responsive neurons were found in the 2 monkeys. Responsive neurons were found at all anterior-posterior levels, but were particularly concentrated in the subicular complex and CA3 subfield. CA1 and CA3, subfields of the hippocampus; DG, dentate gyrus; EC, entorhinal cortex; TF-TH, area in the parahippocampal gyrus. Transverse sections are shown with millimeters posterior to the relative to sphenoid (1) indicated.

showed no response, whereas 62% did not respond in the OA task, and 67% did not respond in the OP task. This suggests that neurons which fire differentially in the FR-DRO task are more likely to respond differentially to other pairs of stimuli in the same task than in the different tasks of OA and OP.

The sites at which these neurons were recorded are shown in Fig. 5. Neurons of the type described were distributed throughout the hippocampal formation and at all anterior-posterior levels. However, there was a tendency for neurons belonging to the subicular complex and to the CA3 subfield to have a relatively high probability of responding in these tasks (5). The neuronal activity recorded from the hippocampus proper could not easily be classified in terms of the "theta" versus "complex spike" types found in subprimate species such as the rat and rabbit. This is consistent with electroencephalographic studies in the primate showing a paucity of theta-like activity (e.g., in humans see Ref. 11) and is discussed in more detail elsewhere (26).

The activity of 239 neurons was analyzed in the different version of the conditional response task, the conditional spatial response task. In this task to obtain a reward the monkey was required to reach to the left side of the monitor screen when one stimulus was shown and to the right side when a second stimulus was shown. This is thus a very clear example of a task in which one spatial response must be learned to one stimulus, and a different spatial response must be learned to another visual stimulus. Of the 239 neurons studied, 120 (50%) showed some response in relation to the task, and 27 (11.3%) neurons responded differentially by responding more to one of the stimulus-motor associations than to the other. A χ^2 test to compare the number of neurons with significant differential responses compared to the number expected by chance gave $\chi^2 = 19.7$ with 1 df and $P < 0.001$. This shows that the differentially responding neurons in this task did not have differential responses just by chance. This proportion of differential neurons is similar to that found in the FR-DRO conditional response task described above and is further evidence that hippocampal neurons have activity which is related to the performance of conditional response tasks.

DISCUSSION

A considerable proportion of hippocampal neurons (14%) had differential activity in the conditional response (FR-DRO) task. In that the learning of this task is impaired by damage to the hippocampal system (8, 9, 29), these neurons may be important in the task. The nature of the function performed by the hippocampus in the task will be considered using the analysis of the factors which accounted for the responses of these hippocampal neurons. Of particular interest is whether these hippocampal neurons had activity which was simply (i.e., unconditionally) stimulus-related, simply movement-related, or instead was related to the formation of associations between stimuli and responses or to the formation of representations useful for such associations to be learned.

For the great majority of the neurons analyzed, their responses could not be accounted for either in terms of stimulus selectivity, nor in terms of movement relatedness. Thus for 45 of 59 neurons (76%) for which data were available, selectivity to the stimuli used in the task could not account for the differential neuronal responses found in the task. For all 129 neurons with differential firing in the conditional response task analyzed in detail, the differential neuronal responses could not be accounted for simply by the movements that the monkey was required to make in a given task. Nor did Go versus NoGo commands for the initiation of a movement account for the responses of the majority of the neurons in that 98% of neurons tested in other tasks such as OA and OP in which Go and NoGo behavior was required did not respond differentially in these other tasks. Similarly, the differential neuronal responses in the conditional response task were not related to factors such as the presentation of reward or licking as shown by the different neuronal activity found in the visual discrimination tasks, OA and OP. These findings thus show that during the performance of conditional spatial response tasks, some hippocampal neurons respond to combinations of the stimulus and the response which the monkey has had to learn to associate so that he can make the correct response to each stimulus. It is of interest that in the human hippocampus, some neurons have been recorded which have activity which alters while a choice of which key to press is being made [for example, in a memory task to indicate whether a stimulus has been seen before (11)]. As in the monkey, the activity of these neurons does not occur simply to the stimulus or to the response (11). The recordings described here suggest that there are neurons in the monkey hippocampal formation which have activity related to the association between stimuli and places in which to respond and the recordings in humans suggest that this could also be the case in humans.

It is important to emphasize that conditional response learning is different to stimulus-reinforcement learning and that the brain mechanisms required for these different types of learning are different. For conditional response learning, an arbitrary association must be learned between a stimulus and a response. The monkey must learn to make one response to one stimulus and a different response to another stimulus, and provided that he does this, he obtains reward on both types of trial, so that simply learning that one stimulus is associated with reward will not help him learn the task. Both stimuli are equally associated with reward. It is for this type of arbitrary mapping of a stimulus to a spatial response that the hippocampus is involved (7, 8, 29). Damage to the hippocampal system does not affect learning in which stimuli are reward related, i.e., the learning of associations between stimuli and primary reinforcers (8, 9). In contrast, amygdala lesions do impair the learning of associations between stimuli and primary reinforcers, so that amygdala lesions do impair performance on tasks in which behavior has to be guided to approach stimuli which have been rewarded previously, or to avoid stimuli which have been associated with punishment previously (7, 8, 19, 22, 29). It is thus suggested that the neurons described here in the hippocampal formation which respond to combinations of a stimulus and a response which the monkey has had to associate together are related to the formation of these arbitrary mappings from a stimulus to a spatial response.

It is also important to emphasize that the conditional response learning being studied here requires flexible asso-

ciations between a stimulus and a spatial response, so that the type of learning is different from the learning of relatively fixed habits. The deficit in conditional response learning produced by hippocampal damage is evident in tests in which either new stimuli are used each day and the monkey has to learn the appropriate response to make to them, or the monkey has on each day to reverse the particular stimulus to response mapping he performed on the previous day (7–9, 29). In this sense it is the flexible and rapid formation of stimulus-response associations which is dependent on the hippocampal system. In contrast, if a habit must be learned that is relatively fixed, for example, to make a given response to a given stimulus consistently from day to day, then this type of relatively slow and persistent learning can still occur in the absence of the hippocampus and the amygdala (18). Instead, this type of motor habit learning may depend on the basal ganglia (18). Now, in the study described here, the relatively flexible and rapid conditional response learning of the type for which the hippocampus is required was investigated, in that the monkeys were often switched to different stimulus pairs for which the response mapping was not well-known to the monkey. Further, in a subsequent study, we are investigating the responses of neurons while the monkey learns conditional response tasks and are finding that the neuronal responses to the stimuli often alter during the learning to produce differential responses of the type described here, so that these neurons are involved in flexible stimulus-response mappings and not just in motor habit learning (6). It is thus suggested that the neurons described here that respond to combinations of a stimulus and a response which the monkey has had to associate together are related to the formation of these flexible arbitrary mappings from a stimulus to a response. Another possible difference between the role of the hippocampus in conditional response learning and that of the basal ganglia in habit formation may be that for the hippocampus, spatial information processing (of where in space the stimulus is or, alternatively, of where in space to make a response) seems to be required for hippocampal damage to reflect impaired performance; that is, a conditional response task with a nonspatial stimulus and a nonspatial response (tapping) is not impaired by hippocampal damage (D. Gaffan, personal communication). Thus it appears that the hippocampus is required when a flexible arbitrary mapping is made from a stimulus to a response, and at least one of these is spatial. In the experiments described here, a spatial response was required (approach to a screen to touch it or withdrawal from the screen), and it is of interest that under these conditions quite a high proportion (14%) of hippocampal neurons had differential activity related to the task. Of course, not all these neurons responded in the same way during this task; that is, the stimulus and response combinations to which the neurons responded were different for the different neurons. This implies that many different associations can be represented by the activity of the whole population of hippocampal neurons; that is, the association memory is efficient (see further Refs. 23, 24).

The experiments described provide some evidence about how the hippocampus may be involved in stimulus-motor response mappings of the conditional, flexible type. The neurons analyzed responded to combinations of stimuli and responses, e.g., to a particular stimulus when it was associated with a particular response, and did not typically respond simply whenever the stimulus was shown or simply (unconditionally) when the response was made. This is consistent with the possibility that associations between the stimuli and the responses were being stored on neurons in the hippocampus (in that the neurons responded to the combinations described). Given that inputs from the visual association cortex (conveying information about the stimulus seen) and from the parietal cortex (probably conveying information about the spatial response being made) reach the hippocampus through the entorhinal cortex and perforant path (35), and that the afferents onto hippocampal pyramidal cells are modifiable by a Hebbian rule (12–14), it is suggested that the synapses onto hippocampal pyramidal cells are one site where synaptic modification involved in flexible stimulus-response association memories are made (23, 24). It will be of interest to investigate in future experiments whether the hippocampal neurons are the first set of neurons in this functional pathway where the associations are made or whether there is sufficient mixing of afferents within, e.g., the entorhinal cortex for such associations to be made there. The available anatomy suggests that inputs from different cerebral cortical areas remain segregated in the entorhinal cortex (2), so that the association between for example information from the parietal and temporal cortices may not be possible in the hippocampal system before the hippocampus proper. Within the hippocampus, it is suggested on the other hand that the possibility for associations to be made between signals originating from widely different areas of the cerebral cortex is present, being implemented for example by the CA3 recurrent collateral system with its Hebb modifiability, which enable associative connections to be made throughout the CA3 cell population (24).

Having formed such associations between stimuli and responses, e.g., onto CA3 neurons, it remains to map these neurons, which simply reflect the memory that there has been an association between a stimulus and a response, into motor space. The responses of the main population of neurons described here are not in motor space, in that they do not show response invariance, i.e., their activity is not unconditionally related to motor responses. However, 6.9% (63 of 905) of the neurons found did have responses which were unconditionally related to movement, occurring simply, e.g., in relation to arm extension. The responses of the stimulus-response combination or association neurons described here could be mapped into motor space via these hippocampal neurons with movement-related activity. Alternatively, the mapping of the stimulus-response combination neurons into motor space might be achieved through the output pathways from the hippocampus to the motor system. One such output pathway consists of connections through the fornix and mammillary body/anterior thalamic nuclei system to the cingulate cortex, and thus via its connections to the supplementary motor area (24).

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REFERENCES

1. AGGLETON, J. P. AND PASSINGHAM, R. E. Stereotaxic surgery under X-ray guidance in the Rhesus monkey, with special reference to the amygdala. *Exp. Brain Res.* 44: 271–276, 1981.
2. INSAUSTI, R., AMARAL, D. G., AND COWAN, W. M. The entorhinal cortex of the monkey. II. Cortical afferents. *J. Comp. Neurol.* 264: 356–395, 1987.
3. BRUNING, J. L. AND KINTZ, B. L. *Computational Handbook of Statistics* (2nd ed.). Glenview, IL: Scott, Foresman, 1977.
4. CAHUSAC, P. M. B., MIYASHITA, Y., AND ROLLS, E. T. Responses of hippocampal formation neurons in the monkey related to delayed spatial response and object-place memory tasks. *Behav. Brain. Res.* In press.
5. CAHUSAC, P. M. B., ROLLS, E. T., AND MIYASHITA, Y. A comparative study of responsive neurons in different subareas of the primate hippocampal formation. *Neurosci. Lett.* 29, *Suppl.*: S103, 1987.
6. CAHUSAC, P. M. B., ROLLS, E. T., MIYASHITA, Y., AND NIKI, H. Modification of the responses of hippocampal neurons in the monkey during the learning of a conditional spatial response task. In press.
7. GAFFAN, D. Hippocampus: memory, habit and voluntary movement, *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 308: 87–99, 1985.
8. GAFFAN, D. Amnesia, personal memory and the hippocampus: experimental neuropsychological studies in monkeys. In: *Cognitive Neurochemistry*, edited by S. M. Stahl, S. D. Iversen, and E. C. Goodman. Oxford, UK: Oxford Univ. Press, 1987, p. 46–56.
9. GAFFAN, D. AND HARRISON, S. Reversal learning by fornix-transected monkeys. *Q. J. Exp. Psychol.* 36B: 223–234, 1984.
10. GAFFAN, D. AND SAUNDERS, R. C. Running recognition of configural stimuli by fornix-transected monkeys. *Q. J. Exp. Psychol.* 37B: 61–71, 1985.
11. HALGREN, E., BABB, T. L., AND CRANDALL, P. H. Activity of human hippocampal formation and amygdala neurons during memory testing. *Electroencephalogr. Clin. Neurophysiol.* 45: 585–601, 1978.
12. KELSO, S. R., GANONG, A. H., AND BROWN, T. H. Hebbian synapses in the hippocampus. *Proc. Natl. Acad. Sci. USA* 83: 5326–5330, 1986.
13. LEVY, W. B. Associative changes in the synapse: LTP in the hippocampus. In: *Synaptic Modification, Neuron Selectivity, and Nervous System Organization*, edited by W. B. Levy, J. A. Anderson, and S. Lehmkuhle. Hillsdale, NJ: Erlbaum, 1985, chapt. 1, p. 5–33.
14. MCNAUGHTON, B. L. Activity dependent modulation of hippocampal synaptic efficacy: some implications for memory processes. In: *Neurobiology of the Hippocampus*, edited by W. Seifert. London: Academic, 1984, chapt. 13, p. 233–252.
15. MERRILL, E. G. AND AINSWORTH, A. Glass-coated platinum-plated tungsten microelectrodes. *Med. Biol. Eng.* 10: 662–672, 1972.
16. MILNER, B. Disorders of learning and memory after temporal lobe lesions in man. *Clin. Neurol. Neurosurg.* 19: 421–446, 1972.
17. MISHKIN, M. Memory in monkeys severely impaired by combined but not separate removal of amygdala and hippocampus. *Nature Lond.* 273: 297–298, 1978.
18. MISHKIN, M. AND PETRI, H. L. Memories and habits: some implications for the analysis of learning and retention. In: *Neuropsychology of Memory*, edited by L. R. Squire and N. Butters. New York: Guildford, 1984, p. 287–296.
19. MURRAY, E. A. AND MISHKIN, M. Amygdalectomy impairs crossmodal association in monkeys. *Science Wash. DC* 228: 604–606, 1985.
20. PARKINSON, J. K., MURRAY, E. A., AND MISHKIN, M. Evidence for the participation of the hippocampus in memory for the location of objects. In press.
21. PETRIDES, M. Deficits on conditional associative-learning tasks after frontal- and temporal-lobe lesions in man. *Neuropsychologia* 23: 601–614, 1985.
22. ROLLS, E. T. Connections, functions and dysfunctions of limbic structures, the prefrontal cortex, and hypothalamus. In: *The Scientific Basis of Clinical Neurology*, edited by M. Swash and C. Kennard. London: Churchill Livingstone, 1985, p. 201–213.
23. ROLLS, E. T. Information representation, processing and storage in the brain: analysis at the single neuron level. In: *The Neural and Molecular Bases of Learning*, edited by J.-P. Changeux and M. Konishi. Chichester, UK: Wiley, 1987, p. 503–540.
24. ROLLS, E. T. Functions of neuronal networks in the hippocampus and neocortex in memory. In: *Neural Models of Plasticity: Theoretical and Empirical Approaches*, edited J. H. Byrne and W. O. Berry. New York: Academic, 1988.
25. ROLLS, E. T., BURTON, M. J., AND MORA, F. Hypothalamic neuronal responses associated with the sight of food. *Brain Res.* 111: 53–66, 1976.
26. ROLLS, E. T., MIYASHITA, Y., CAHUSAC, P. M. B., KESNER, R. P., NIKI, H., FEIGENBAUM, J., AND BACH, L. Hippocampal neurons in the monkey with activity related to the place in which a stimulus is shown. *J. Neurosci.* In press.
27. ROLLS, E. T., PERRETT, D. I., CAAN, A. W., AND WILSON, F. A. W. Neuronal responses related to visual recognition. *Brain* 105: 611–646, 1982.
28. ROLLS, E. T., SANGHERA, M. K., AND ROPER-HALL, A. The latency of activation of neurones in the lateral hypothalamus and substantia innominata during feeding in the monkey. *Brain Res.* 164: 121–135, 1979.
29. RUPNIAK, N. M. J. AND GAFFAN, D. Monkey hippocampus and learning about spatially directed movements. *J. Neurosci.* 7: 2331–2337, 1987.
30. SCOVILLE, W. B. AND MILNER, B. Loss of recent memory after bilateral hippocampal lesions. *J. Neurol. Neurosurg. Psychiatry* 20: 11–21, 1957.
31. SMITH, M. L. AND MILNER, B. The role of the right hippocampus in the recall of spatial location. *Neuropsychologia* 19: 781–793, 1981.
32. SQUIRE, L. Mechanisms of memory. *Science Wash. DC* 232: 1612–1619, 1986.
33. SQUIRE, L. Complex connections involved in memory functions. In: *Neural Models of Plasticity: Theoretical and Empirical Approaches*, edited by J. H. Byrne and W. O. Berry. New York: Academic, 1988.
34. SQUIRE, L. R. AND ZOLA-MORGAN, S. The neurology of memory: the case for man and non-human primate. In: *The Physiological Basis of Memory* (2nd ed.), edited by J. A. Deutsch. New York: Academic, 1983.
35. VAN HOESEN, G. W. The parahippocampal gyrus. New observations regarding its cortical connections in the monkey. *Trends Neurosci.* 5: 345–350, 1982.
36. WOODWARD, R. H. AND GOLDSMITH, P. L. Cumulative sum techniques: mathematical and statistical techniques for industry. Edinburgh: Oliver & Boyd, 1964 (ICI Monogr. 3).