inhalation rates. The activity maxima and minima thus do not simply reflect some fixed latency from onset of nasal flow to onset of excitation or inhibition. Also, although the sharpness of the peaks and troughs in the ICH are related to the concentration of an effective odor, the timing of the peaks and troughs generally is not. In addition, the activity patterns during the inhalation cycle remain stable and reproducible for several hours. (In contrast, the overall firing frequency can spontaneously undergo as much as two- to threefold changes over these extended periods.) These three types of invariance would be expected if the observed activity patterns during the inhalation cycle were involved in specifying and monitoring the olfactory environment under both resting and active conditions.

Our results suggest that changes in the overall firing frequency of units in the olfactory bulb provide neither a necessary nor a sufficient indication of unit responsiveness to specific odor stimuli. The effect of stimuli upon unit activity within the inhalation cycle must also be considered. The use of periodic inhalation cycles in electrophysiological studies preserves a characteristic feature of normal olfactory stimulation. The regular and periodic patterning of nasal air flow may itself play a role in the processing of neural information about odor quality. Although the neural structures responsible for stimulus-specific modulation of activity of olfactory bulb units have not been identified, the many recurrent pathways of the bulb itself (*8*) and the centrifugal inputs from at least three separate (and more caudal) regions of the limbic forebrain (*9*) are likely candidates.

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3. Compressed breathing air from a standard air cylinder was passed through a calibrated reducing valve, dried with $CaSO_4$, filtered through charcoal, heated to 25°C by passage through coils in a water bath, and rehumidified. The total flow was then divided into two main streams. The first was used to provide the background (clean air) flow. It was passed through a calibrated flowmeter and valve to a mixing chamber and nose cone that was fitted loosely around the snout of the experimental animal. The other stream was divided by parallel valves and flowmeters into equal substreams. Some of the substreams were passed through a group of large jars containing stimulus objects or animals. One of these jars remained empty. The jars rested on a heated platform that maintained their interior temperature constant at about 25°C. The other substreams passed through t-connectors whose stems were fitted to syringes containing pure chemical vapors. The vapors were injected into the latter substreams by an infusion pump. All substreams then were passed to a distribution valve system. Odor-bearing substreams were normally ported to a remote waste receptacle containing activated charcoal. A stimulus delivery line led from the distribution valve system to the mixing chamber and nose cone, and under background conditions received the substream from the empty jar. Stimuli were presented by a manual switch that simultaneously removed the substream of the empty jar from the stimulus delivery line and replaced it with a preselected, odor-bearing substream. Thus, except for a brief switching transient, there was no net change in air flow to the mixing chamber and nose cone during stimulus presentation. To terminate a stimulus presentation, the manual switch was reversed, so that the substream of the empty jar was returned to the stimulus delivery line. The concentration of an odor arriving at the nose cone could be controlled by varying the ratio of air flow in the stimulus delivery line to that in the main clean air stream. The concentration of pure chemical odors could be controlled further by varying the rate of the infusion pump.
4. Glass capillary microelectrodes were drawn to a tip diameter of less than 1 μ and were filled with 2*M* NaCl saturated with fast green FCF. Iontophoretic injection of the dye after a recording session aided in locating the recording site [R. C. Thomas and V. J. Wilson, *Nature* **206**, 211 (1965)]. Amplified unit potentials were displayed on a cathode-ray oscilloscope and recorded on magnetic tape along with other relevant data. Histograms were constructed by analyzing the tape recording with the aid of a spike discriminator and small digital computer.
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Theta Rhythm: A Temporal Correlate of Memory Storage Processes in the Rat

Abstract. *We examined the amount of theta rhythm (4 to 9 hertz) in cortical electroencephalograms of rats for 30 minutes after training in one-trial tasks. Some animals received electroconvulsive shock after training. The amount of theta in the electroencephalogram after training was positively correlated with the degree of subsequent retention of a footshock, whether animals had received electroconvulsive shock or not.*

Numerous studies have attempted to find electroencephalogram (EEG) correlates of learning (*1*). In a summary of research published prior to 1961, Morrell noted that a hypersynchronous cortical EEG wave form in the range of theta rhythms (4 to 9 hz) was associated with the early stages of conditioning in carnivores and concluded that such waves might be correlated with the "inscribing of an experience into neural structure" (*2*).

Although they are most prominent in the hippocampus (*3*), theta waves are readily recorded from many regions of the brain, including cortex, when rodents and carnivores are alert or aroused, and during the early stages of conditioning (*4*). Thus, the hypersynchronous cortical correlate of conditioning appears to be a cortical theta rhythm.

Adey and his associates (*5*) have proposed a critical role for theta rhythms during memory consolidation. They suggest that long-term changes in the pattern of theta rhythms might reflect the consolidation of specific information over extended periods of conditioning. However, it may be that theta rhythms merely reflect the activation of brain processes which are related to, or possibly involved in, memory storage (*6*). In this view, a simple relationship might exist between the amount of theta during the period after

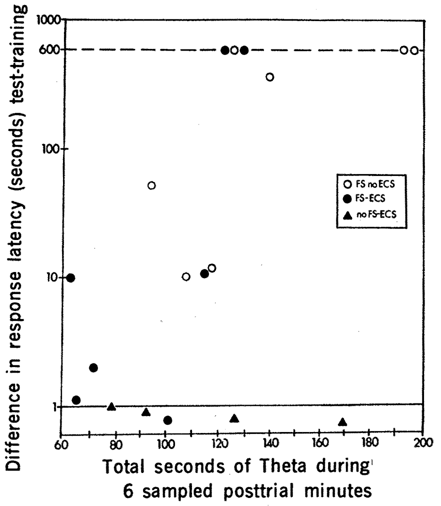

Fig. 1. Relation in individual subjects between amount of posttrial theta and degree of retention 2 days after training. Amount of theta was calculated by sampling the EEG at specific intervals (*8*). The number of seconds of theta were counted for each animal during posttraining minutes 4 and 5, 14 and 15, and 29 and 30.

training (posttrial period) and memory as measured on a subsequent retention test. We tested this possibility by examining the temporal relation between theta and memory storage in one-trial learning tasks similar to those generally used in the study of posttrial memory storage processes (*7*). The findings indicate that the amount of posttrial theta is an EEG correlate of time-dependent consolidation processes, as the latter are generally operationally defined (*7*).

We surgically implanted four cortical screw electrodes, two posterior (2 mm anterior to lambda, 2 mm lateral to midline) and two anterior (1 mm posterior to bregma, 2 mm lateral to midline) bilaterally in 18 male Wistar rats (70 days old). The screws were connected to miniature electrical connectors. With this method, we can readily obtain theta by recording between one anterior and one posterior cortical electrode. Recording between two posterior screws yields attenuated theta because of the amplifier's rejection of similar EEG patterns.

Recordings were made on a four-channel (Grass model 7) polygraph and on an FM tape recorder (Vetter). Analysis of EEG data was performed visually by using statistical sampling methods (*8*). Some data were also analyzed by computer (*9*).

In the first experiment, three groups of rats were trained in a one-trial, step-through inhibitory avoidance task (*10*). Rats were initially connected to the recording cable and placed into a lighted transparent compartment (10 by 20 by 35 cm). They then stepped into a black, opaque compartment (30 by 30 by 35 cm) containing a grid floor. When the hind feet of the rat were in the dark compartment, a footshock (FS) of 1 ma was given until the rat escaped back into the lighted compartment. A switching circuit disconnected the rats from the recording apparatus during FS and permitted direct administration of electroconvulsive shock (ECS) to all four cortical screws. The anterior and posterior screws of each hemisphere were short-circuited.

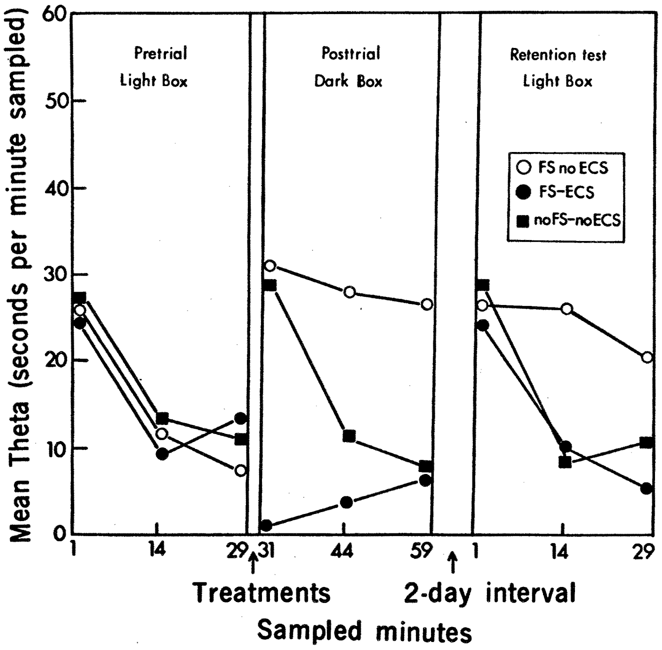

Fig. 2. Temporal changes in occurrence of EEG theta rhythm in three groups of rats as a function of a single FS and presence or absence of ECS. The number of seconds of theta was counted for each animal during minutes 1, 14, and 29 of each 30-minute session of EEG records (*8*).

Rats in one group of animals (FS-no ECS, $n = 7$) were allowed to step through, received FS, and were then placed in a holding cage for 30 minutes. Rats in a second group (FS-ECS, $n = 7$) stepped through, received FS, were removed to the holding cage, and 10 seconds later received ECS (15 ma, 1 second, 60 hz) through the cortical screws. Rats in the third group (no FS-ECS, $n = 4$) stepped through but did not receive FS. They were then placed in the holding cage and received ECS 10 seconds after stepping through. Records of EEG were obtained from all animals in the holding cage for 30 minutes after the treatments. Two days after training all animals were placed back into the training apparatus and step-through latencies were again measured. The rats were allowed a maximum of 600 seconds in which to step through on the retention test. No EEG records were taken on the test. The difference between the initial step-through latency and the test latency was used as the measure of retention.

The total number seconds of theta which were present during the 6 minutes sampled in the 30-minute posttrial period for individual subjects and the retention scores (Fig. 1) indicate that for rats given FS, the retention latencies were highly correlated with the amount of posttraining theta. For the FS-no ECS and the FS-ECS groups combined, the Spearman correlation coefficient (*11*) was +.88. The ECS significantly lowered the amount of posttrial theta. However, individual animals that received FS-ECS, but exhibited a substantial recovery of theta after ECS, exhibited good retention 2 days later. Conversely, individual animals that did not receive ECS after FS, but still showed low posttrial theta scores, exhibited low retention scores 2 days later.

Thus, the amount of posttrial theta was a better predictor of subsequent retention than was the presence or absence of ECS. The primary effect of ECS appeared to be to lower the probability of theta during the posttraining period. In the control group (no FS-ECS) a recovery of theta after ECS did not lead to high retention latencies; the relationship between posttrial theta and retention latencies was seen only in rats given FS training.

The mean of the cortical frequency (*8*) was also correlated with retention scores but these correlations were smaller and were not significant. The findings of the first experiment indicate that theta rhythms are present during the period after training. However, the findings do not indicate the temporal course of theta rhythms during the posttrial period. In a second experiment we examined this question, as well as the possibility that the amount of theta rhythm might also be used as an index of retention.

We divided the animals into three new groups of six subjects and balanced the groups for prior training. We gave them habituation trials in two wooden boxes (30 by 30 cm) containing grid floors. One box was black and one was white. Habituation trials lasted 30 minutes in each box (counterbalanced sequence) for 3 days. On day 4, each animal was placed in the white box and EEG records were obtained for 30 minutes (pretrial period). One group (FS-no ECS) then received a FS (1 ma, 2 seconds) and was immediately placed in the black box for 30 minutes (posttrial period). Animals in a second group (FS-ECS) were then given ECS (10 ma, 1 second) 5 seconds after the FS and placed in the black box for 30 minutes. The third group (no FS-no ECS) received neither FS nor ECS. Rats in the latter group were placed directly into the black box after the initial 30-minute period in the white box. On day 6, 2 days after training, we returned all animals to the white box and recorded EEG for 30 minutes (retention test period).

Apparently any change in surroundings is sufficient to produce high theta activity (Fig. 2). However, the theta increases habituate rapidly over a 30-minute period (pretrial). A significant event, such as FS, can maintain theta at high levels for at least 30 minutes after the event (FS-no ECS, posttrial period). The ECS after FS produced a significant (*8*) depression in theta rhythms followed by gradual recovery over the 30-minute period (FS-ECS group, posttrial period). These posttrial differences in theta were all statistically significant (*8*), despite the small group sizes.

On the retention test, animals in the FS-no ECS group again exhibited high theta for the entire 30 minutes, whereas animals in the FS-ECS and the no FS-no ECS groups exhibited the same theta-habituating pattern shown during the pretrial period. Using EEG patterns as the measure of retention, we found that the performance of animals in the FS-ECS group was similar to that of the no FS-no ECS group: neither showed evidence of retention of the FS. However, animals in the FS-no ECS group exhibited significantly higher theta scores on the 30-minute retention test than did those in the no FS-no ECS group, which indicates retention of the FS.

In short, under our conditions when consolidation processes can be presumed to be active (for example, FS-no ECS) theta is increased; when consolidation is experimentally decreased (FS-ECS) theta is decreased. Further, the amount of theta in the EEG can also apparently be used as a measure of retention, at least under these one-trial training conditions.

We also noted in the first experiment that ECS 10 seconds after the treatments produced two types of seizure patterns. One consisted of monophasic "spikes" beginning 20 to 30 seconds after ECS with a frequency of one to two per second (*12*), whereas the other pattern consisted of large, slow waves (200 mv) of a similar frequency and latency. Only the spike pattern was associated with amnesia and with suppression of theta in the FS-ECS group. It is possible, therefore, that seizure-produced suppression of theta is an important factor in understanding the reported correlation (*13*) between amount of seizure activity and amount of subsequent amnesia. Hippocampal EEG has also been reported to be abnormal for up to several hours after ECS (*14*).

In the first experiment, the return of theta after ECS was associated with good retention. This finding may be related to other evidence that memory consolidation can be "restarted" by various stimulant treatments given after the administration of amnesia-producing treatments (*15*). It is possible that treatments effective in restarting consolidation might be acting by reactivating theta rhythms before some short-term memory process (*16*) has fully decayed.

These findings indicate that in rats, the degree of retention of a one-trial training experience varies with the amount of theta rhythm activity recorded during the period after training. Under these conditions, theta may be a correlate of a brain state which is optimum for memory storage.

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8. All reported results are significant at $P < .05$ (two-tailed). During all sampled minutes, theta and the mean frequency of the electrocorticogram were both analyzed. In each experiment the EEG's of all animals were analyzed during the same minutes in order to allow statistical comparisons to be made (see figures to determine which minutes were sampled in each experiment). Theta was scored for presence or absence during each second of any sampled minute. The number of seconds during which theta rhythms were prominent represented the theta score for that sampled minute. A second was considered to contain theta if more than half of the second was dominated by synchronous waves within the theta frequency range (4 to 9 hz in rats). Mean of cortical frequency was obtained by counting the number of waves per second every sixth second during a sampled minute. The mean of the ten frequency values was taken as the mean of cortical frequency for an animal during that sampled minute. In all instances, Mann-Whitney U tests (*11*) were used for comparing group differences during any sampled minute.
9. A computer program for the analysis of EEG data on PDP8 and PDP12 computers has been developed at the University of California, Irvine, by H. Birch and P.W.L. Some of the data from the first experiment were analyzed by computer. The results support the conclusions that were obtained by visual analysis.
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