distilled water was used. No airborne organisms were isolated from the vicinity of the pond in repeated tests with the Andersen sampler.

To see whether the isolates from the Don Juan Pond were peculiar to the environment or whether they were also found in freshwater or less saline ponds in nearby valleys, collections were made, in sterile plastic bottles, from meltwater on the edges of Lake Bonney, in Taylor Valley, and Lake Vanda, in Wright Valley—lakes which are permanently covered with ice. Samples were also obtained from the 60-m level of Lake Vanda. The temperatures and results of chemical analyses of these lakes are shown in Table 2 (*10*).

From standard dilution plates of each meltwater sample prepared with plain nutrient and peptone glucose-acid agars, incubated at 22°C, four types of bacteria were isolated. These were Gram-negative and Gram-positive nonmotile rods and Gram-positive cocci. A yeast growth form was obtained from Lake Vanda; the yeast *Sporobolomyces* was isolated only from Lake Bonney. No organisms were isolated from the 60 m level of Lake Vanda. None of the organisms from Lake Vanda and Lake Bonney appear to be the same as those isolated from the saline Don Juan Pond.

The Don Juan Pond provides an unusual ecological picture. A distinctive relative concentration of ions results in a high salt content. The aerobic microbial population is restricted in natural habitat and is not found in nearby waters. Yet in the laboratory these microbes can be cultured over a wide range of salt concentrations, temperatures, and nutrients. Their habit of growing in colonies, as observed in direct examination of the natural habitat, is retained in laboratory culture in broth of varying salinity and incubation temperature. Our preliminary studies indicate a possible relationship between high salt concentration and the ability to grow at low temperatures and in an environment of low organic content (*11*).

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Hippocampal Ablation and Passive Avoidance

Abstract. Six rats with bilateral ablation of the hippocampus and six rats with extensive destruction of the neocortex were trained to enter a small compartment, while hungry, for a food reward. After 35 trials, spread over 4 days of training, the animals were given a shock while they were eating in the goal box. After the shock the rats with cortical lesions would not enter the goal compartment on the remainder of the trials given on the same day and only gradually began to re-enter over the next 2 days. The effect of the shock on the subjects with hippocampal ablation was slight and transient, suggesting that the ability to make passive avoidance responses was impaired.

An anatomical dissociation of the abilities necessary to acquire active and passive avoidance responses has been indicated by recent work of McCleary (*1*). Cats with lesions beneath the genu of the corpus callosum had difficulty in acquiring a passive avoidance response, but their ability to acquire an active avoidance response seemed unimpaired. Animals with lesions of the cingulate cortex showed just the opposite deficit. Since rats with radical bilateral lesions involving from 50 to 90 percent of the hippocampus have no difficulty in acquiring an active avoidance response (*2*), we designed a study to determine whether such lesions would interfere with their acquiring a passive avoidance response.

Twelve rats of the Sprague-Dawley strain were used as subjects. When the rats were about 90 days old, radical bilateral hippocampal ablation was carried out in six of the animals and extensive neocortical lesions were made in the other six. The surgical techniques used were similar to those described by Isaacson, Douglas, and Moore (*2*). About 2 weeks later the animals were put on a 23-hour food deprivation schedule; this was maintained throughout the experiment. During the experiment the body weights of the subjects were maintained at 85 percent of the preoperative weights. All the subjects were given experience, prior to the experiment, in learning to seek food rewards in a maze. The experiment was begun when the animals were about 120 days old.

The apparatus consisted of two compartments separated by a guillotine-type door. The larger compartment had unpainted wood walls and floor and a lid of transparent plastic. The floor area of this compartment was 12 by 18 inches; the height was 10 inches. The smaller compartment, connected to the larger compartment by a passageway, had wooden walls, a copper grid floor, and a wire mesh top. The floor area of this smaller compartment was 6 by 4 inches; the height was 4 inches. A small copper tray about 1 by ¾ by ¼ inch was attached to that end of the smaller compartment farthest away from the passageway. Food rewards in the form of wet mash made from food powder were given in this tray during training.

The animals were allowed to explore the apparatus for half an hour on the day prior to the commencement of training. Every day at the start of the training period the subject was placed in the larger compartment for several minutes while the door leading to the smaller compartment was closed. Then the door was opened and the animal could enter the smaller compartment and receive, in the feeding tray, a small amount of the wet mash. While the rat was eating in the smaller compartment some of the food was placed in the larger compartment as a lure to induce it to leave the smaller compartment and return to the larger one. As the animal re-entered the larger compartment, the guillotine door was closed. The amount of food given the animal in the smaller compartment was always greater than that given it in the larger compartment. Between 20 and 60 seconds after the animal had eaten

the food in the larger compartment the door leading to the smaller compartment was again opened. On every trial the animal was allowed 30 seconds to enter the smaller compartment. If it did not enter the smaller compartment in that time the door was closed and the trial was counted as a trial with the maximal period of latency, 30 seconds.

All the subjects were given ten training trials each day for 6 days. On the fifth trial of the fourth day a shock was administered to the feet of the animals through the copper grid floor of the smaller compartment while they were eating there. All the animals received shocks of the same intensity [0.52 ma (alternating current)]. Shock of this intensity was sufficient to drive the animals out of the smaller compartment. The door connecting the compartments remained open for 15 seconds, and electrification of the floor continued during this period. If an animal attempted to re-enter the smaller compartment in this interval it received another shock. No difference in the number of these immediate re-entries was found for the two groups of animals. Fifteen seconds after initial electrification of the grid the connecting door was closed and the shock was turned off. No subsequent shocks were given in the course of the experiment. The sixth trial of the fourth day began 1 minute after the connecting door had been closed after the shock trial. The animals continued in training for 2 days after the day on which shock was administered.

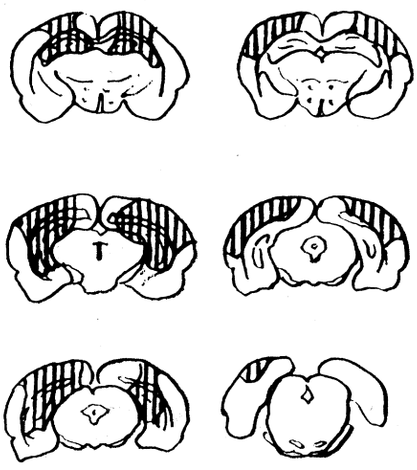

Fig. 1. Tracings of representative brain sections from animals with hippocampal and neocortical lesions. The three sections at left are from an animal in which the hippocampus and overlying neocortex had been destroyed. The sections at right are from an animal in which only the neocortex had been damaged. Shaded areas indicate lesions.

After the completion of training the animals were sacrificed. The bodies were perfused intracardially with Formalin; the brains were recovered and embedded in paraffin. Sections were cut at 20-μ intervals through the lesion, and every tenth section was retained and stained with thionin. Histological examination of the slides indicated destruction of approximately 70 percent of the hippocampal formation and minor injury to the thalamus and other nearby subcortical areas in the rats with hippocampal lesions. The proportion of neocortex destroyed in the animals with neocortical lesions was greater than the proportion of hippocampus destroyed in the rats with hippocampal lesions, as a result of our attempt to destroy equal amounts of brain tissue in rats of the two groups. Tracings of three representative brain sections from a rat of each group are shown in Fig. 1.

Animals with either kind of lesion readily learned to enter the smaller compartment when the door was opened. The shock given in the smaller compartment on the fourth day produced dramatically different effects on subjects of the two groups. Every animal with hippocampal lesions re-entered the smaller compartment on the first trial that followed the shock, whereas not one of the animals with cortical lesions entered the smaller compartment even on the second or third postshock trial. Only two entries were made by an animal of the cortical-lesion group in the five postshock trials; both of these were made by the same animal after extended periods of latency. Median values for latency of entry and the range of latencies for the two groups on all trials are shown in Fig. 2. The prolonged effect of the single shock may be seen in the long latencies of the animals with cortical lesions, as compared with the small and transient effect of shock on the animals with hippocampal lesions. Further, it may be seen in Fig. 2 that latency of entry was shorter and less variable early in acquisition training in the hippocampal-lesion group. This finding is comparable to the finding (*2*) of rapid acquisition of an active avoidance response in a shuttle box by rats with hippocampal lesions.

In interpreting these data one must recognize the possibility that the behavioral effects of the "hippocampal" lesions may be produced by destruction of both hippocampal and neocortical tissues. The behavior of the animals with cortical lesions indicates that damage to overlying neocortex alone will not produce such effects. That the hippocampus itself may be important for the mediation of passive avoidance behavior is suggested by a study by Kimura, who found that animals with small electrolytically induced lesions of certain parts of the hippocampus tended to exhibit short-latency approach responses in a straight-alley situation despite a shock at the food cup (*3*). The animals in his study received only minimal damage to the neocortex. Our results, as well as those of Kimura, suggest that lesions of the hippocampus may impair an animal's ability to exhibit passive avoidance behavior.

Kaada, Rasmussen, and Kveim have just reported a study in which drinking in certain control animals which had relatively small lesions of the hippocampus was only slightly inhibited by electrical shock (*4*). The difference in the results of their study and of ours could stem either from the greater amount of hippocampal damage suffered by the animals of our study or from the

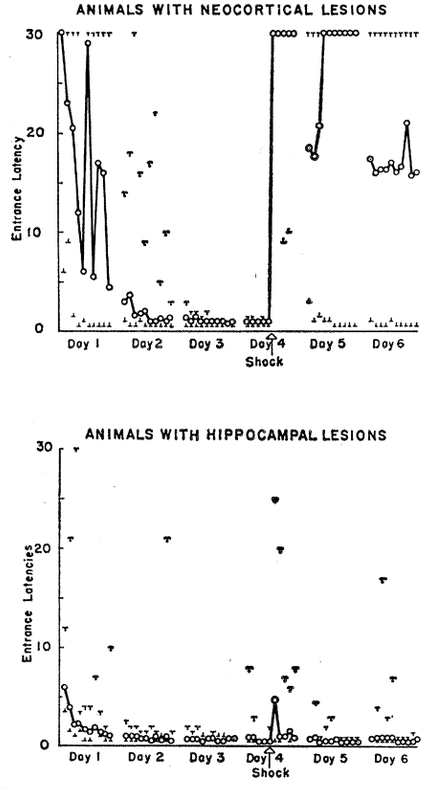

Fig. 2. Graphs of median values, and range, for latency of entry for animals with neocortical lesions and animals with hippocampal lesions. (Circle) Median value; (T) upper value for latency; (⊥) lower value for latency.

differences in experimental procedures in the two studies. In our study the inhibition was of a learned approach response rather than of simple drinking behavior (*5*).

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Extreme Spindles: Correlation of Electroencephalographic Sleep Pattern with Mental Retardation

Abstract. An electroencephalographic abnormality is described which is relatively common among children below 12 years of age who are mentally retarded. This pattern consists of exaggerated sleep spindles, which are of higher voltage and more continuously present than in normal persons, and it correlates specifically with mental retardation and not with epilepsy or cerebral palsy.

Numerous workers have sought unsuccessfully to find features of the electroencephalogram (EEG) which correlate specifically with mental retardation. Our recent analysis of the waking and sleeping electroencephalograms of 1118 mentally retarded persons without clinical epilepsy failed to reveal such patterns (*1*). However, one of us, (E.L.G.) decided that because the epileptic disorder which correlates most highly with mental retardation, namely hypsarhythmia, is characteristically a disorder of infants and disappears later in childhood (*2–4*), it would be worth while to look intensively at the youngest age group. It has been our standard practice for a number of years to record the EEG in subjects both when they were awake and when they were asleep (*3*). A review of the sleep recordings of young, mentally retarded patients revealed a strikingly distinctive pattern which has been termed *extreme spindles* because it appears as an exaggeration of normal sleep spindles (Fig. 1). The pattern shows a frequency of from 8 to 15 cycles per second and is of much higher voltage and is more nearly continuous than normal spindle activity. In the most severely retarded patients the spindles are continuous. They are diffuse and quite commonly they are seen even in the waking state, though when the patient is awake they are of low to medium voltage and are mixed with waking activity. This abnormality is most usually found among mentally retarded children below the age of 5 years; it has not been observed in any child of more than 12.

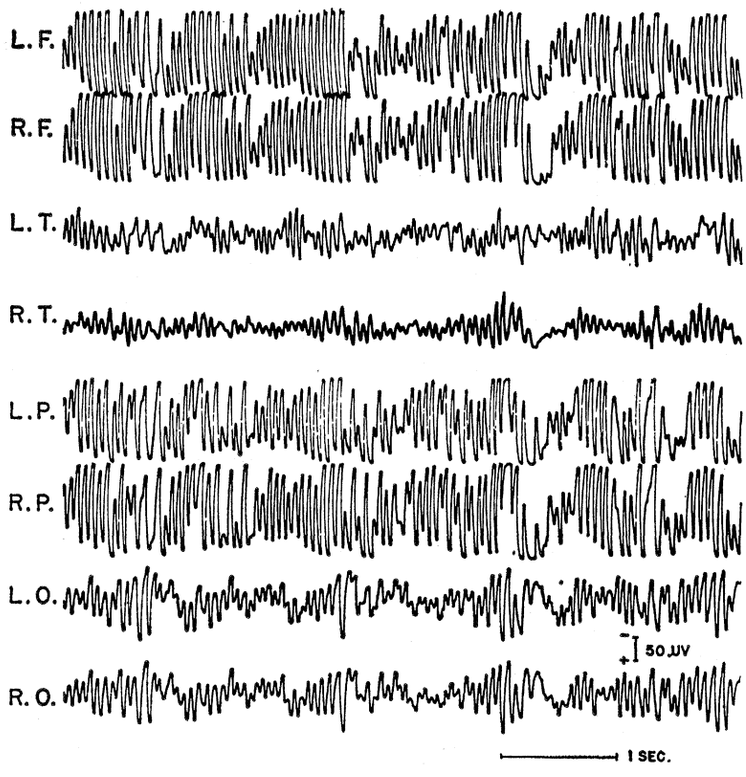

Fig. 1. Sleep recording of young, mentally retarded patient showing extreme spindles.

Extreme spindles do not correlate with epilepsy (*3*, *4*). That they are an abnormality is indicated by the fact that they have been found in only 1 out of more than 3000 control subjects below 12 years of age. A preliminary survey indicates that 70 to 80 percent of children with this pattern are mentally retarded. The pattern was found in 17 percent of 300 noninstitutionalized, mentally retarded children below the age of 8 years. It occurs in 20 percent of cerebral palsy patients classified as ataxics, in 16 percent of athetoids, but in only 6 percent of spastics. Thus extreme spindles are associated with damage to the extrapyramidal rather than the pyramidal system.

There is some suggestive evidence that spindles may be initiated by impulses originating in the intralaminar nuclei of the thalamus (*5*). It seems possible that extreme spindles may be caused by a destructive or irritative lesion. Lindsley has suggested that damage to the reticular formation may be responsible for some types of mental retardation (*6*). Our own studies (*7*) and those of other workers show that high voltage, fast activity, and exaggerated spindles, somewhat like those we have classified as extreme spindles, can be produced by drugs. This pattern may be caused by a metabolic defect and, of course, a combination of structural and metabolic factors may be responsible. A metabolic defect might damage the reticular formation or some other area which regulates the fast activity that appears during sleep.

An immense number of reports have been published relating directly and indirectly in these findings. Literature references can be more meaningful and their significance more properly assessed when our studies have progressed further. The bibliography merely traces our own line of investigation.

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