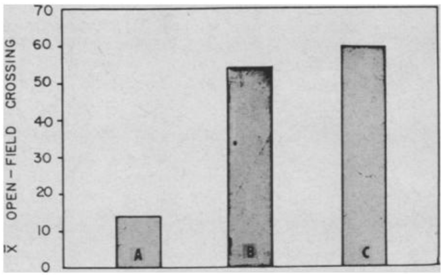

Fig. 2. Effect of grouping and prior housing conditions on locomotor activity. *A*, isolates; *B*, density 10 (prior grouping); *C*, density 10 (prior isolation).

at regular intervals on the floor area. The entire arena was enclosed by walls about 12.7 cm high. The floor and barriers were painted gray and the smaller squares were marked off in black. The animal was placed in one corner, a timer started, and the number of squares crossed during a 3-minute period recorded. Crossing a square was defined as placing both forepaws over a line. Twenty-four hours later the mice were killed, and their adrenals and testes were removed and weighed to the nearest 0.1 mg.

Animals previously living in groups of five showed no overt fighting during the half-hour observation when grouped in a single cage. Animals previously isolated prior to grouping began to fight after the first 10 minutes. Seventy-eight individual encounters were noted and nearly one-third of the half-hour period was spent in fighting. One hour later all fighting had ended but all animals of both groups were active. Although animals previously isolated remained more active during the second hour after grouping, by the third hour the groups could not be distinguished on the basis of activity, and by the sixth hour and thereafter animals of both conditions huddled inactive in one section of the cage.

Adrenal and body weight did not vary with conditions of housing, but testis weight showed a decline with grouping ($F = 3.88$; df = 2 and 27; $p < .05$), the greatest decline occurring with those animals having been isolated prior to grouping ($p < .01$). These values are set out in Fig. 1. The greater sensitivity of the testes to grouping is in accord with previous data (*2*).

Open field activity also varied with condition of housing and in the predicted direction. Animals from both populations were more active than animals living individually ($F = 8.43$; df = 2 and 27; $p < .01$) and grouped animals previously isolated were somewhat higher than the other two conditions. These relations are seen in Fig. 2.

A consistent pattern of results is evident from these data; isolation prior to grouping leads to intense fighting, testicular atrophy, and high levels of locomotor activity. It is possible that inattention to similar differences in living conditions preceding increases in density can account for varying results reported in the literature. The possibility exists that the level of aggression is the mediating factor regulating associated changes. Moreover, strict control over the experience of the animals can provide greater predictability for studying relations between population density, endocrine function, and behavior (*5*).

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Timing Behavior after Lesions of Zona Incerta and Mammillary Body

Abstract. *Rats with mammillary body lesions produced with an angled approach do not show a "scalloping" in their behavioral records during fixed-interval reinforcement. Control experiments suggest that this effect was due to the electrode piercing the zona incerta, implicating the participation of extrapyramidal structures in timing behavior.*

In previously published studies (*1*) we have shown that lesions in the septum and hippocampus did not prevent the appearance of the "scallop" in the behaviorial record when rats pressed a bar for food or water under a fixed-interval reinforcement contingency. In fixed-interval reinforcement, reward is delivered only if the animal makes a bar press at a fixed period of time after the previous reward. The "scallop" which reflects the distribution of responding during the fixed interval has been used as evidence for temporal discrimination (*2*, *3*) and has been shown to be unaffected by drive or motivational conditions, with only the total output of bar-pressing behavior being affected by such variables (*1*, *3*). The present report is an extension of these investigations and is concerned with the effects of lesions in the mammillary body and adjacent structures on timing behavior.

After preliminary training in bar pressing for food reward eight rats were prepared with electrolytic lesions 1 mm in diameter in the mammillary body. Two stereotaxic approaches were used in making these lesions. For half the animals a vertical approach through the midline was used, while for the remainder an angled approach aimed at the same focus as the vertical was used and served as a control for damage to midline structures, thereby preserving effects common to the mammillary body lesion. After the experiment the animals were killed, serial frozen sections were made and stained with cresyl violet, and the lesion location and extent were determined.

Seven days postoperatively all animals were started on a training schedule in which the delivery of a 45-mg food pellet was dependent on the animal making a bar press after a minimum interval of 1 minute after the delivery of the previous reward. This training on the 1-minute fixed-interval schedule lasted for 9 days.

Figure 1 consists of typical examples of the cumulative bar-pressing records of the animals with mammillary body lesions. The slope of these curves reflects the response rate, while the vertical deflection indicates the occurrence of reinforcement. The high rates of response shown on these records indicate that regardless of the approach, whether vertical or angled, the mammillary body lesion did not interfere with or prevent bar-pressing behavior. Specifically, on day 1 of the training, the animals with the vertically oriented lesions made approximately 36 responses per reinforcement, while the animals with the angled lesion made, on the average, 40 responses per reinforcement. These behavioral outputs compare favorably with what is obtained by normal nonoperated animals (40 responses per reinforcement) and suggest that the mammillary body lesions did not produce a general behavioral deficit.

A closer examination of these behavior records indicates that on day 1 of the training there was a relatively constant output of bar pressing with no changes of response rate correlated with occurrence of reward. By the final day of training, day 9, a major difference related to the angle of the lesion exists. For all of the animals with the vertically oriented mammillary body lesions the typical fixed-interval scallop has appeared in the behavioral records. Thus for these animals the bar-pressing behavior has adjusted itself to the temporal occurrence of the reward. However, for the animals with the angled mammillary body lesions, the bar-pressing behavior is erratic and shows several different topographies with no consistent correlation of bar pressing with the temporal patterning of reward.

Since the animals with the vertically oriented mammillary body lesion showed a "scallop" in their behavioral records it seems that this structure as well as the septum and hippocampus (*1*) is not critically involved in this behavior. The fact that the animals with the angled mammillary body lesions were unable to learn the fixed-interval schedule emphasizes the importance of effects due to the passage of an electrode.

The failure by all four of the animals with the angled mammillary body lesions to learn the fixed-interval schedule prompted us to carry out additional experiments with lesions in structures which lie along the path of the angle approach and through which the electrode track may have passed. In this way we hoped to identify the anatomical basis for our initial observations. These additional lesions and the resultant behavior are illustrated in Fig. 2. The behavioral records are typical samples taken from the ninth day of training. As Fig. 2 shows, lesions in fimbria, cerebral peduncle, mammillary peduncle, medial lemniscus, substantia nigra, or cortex did not prevent the appearance of the scallop during the fixed-interval schedule. Lesions in zona incerta, however, produced a behavior deficit that was qualitatively similar to that produced by the angled mammillary body lesion. These additional results suggest that the failure to learn

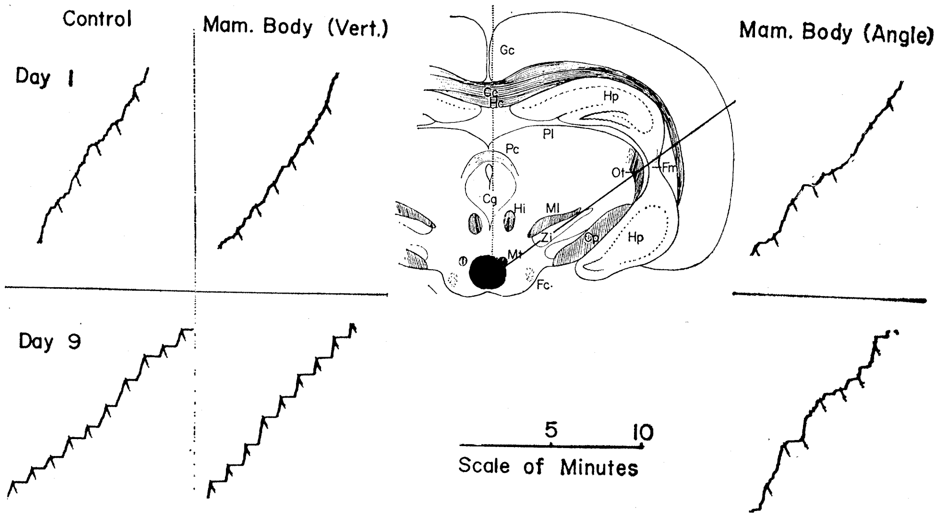

Fig. 1. The mamillary body lesion and the two stereotaxic approaches used for the experimental groups. Typical behavior records for the vertical and angled approaches are shown to the left and right of the lesion diagram, respectively. One and 9-day training records are compared with those of nonoperated controls. Abbreviations: *Cc*, corpus callosum; *Cg*, central gray; *Cp*, cerebral peduncle; *Fc*, fornix column; *Fm*, fimbria; *Gc*, gyrus cinguli; *Hc*, hippocampal commissure; *Hi*, habenulointerpeduncular tract; *Hp*, hippocampus; *Ml*, medial lemniscus; *Mt*, mammillothalamic tract; *Ot*, optic tract; *Pc*, posterior commissure; *Pl*, n. postero-lateralis; and *Zi*, zona incerta.

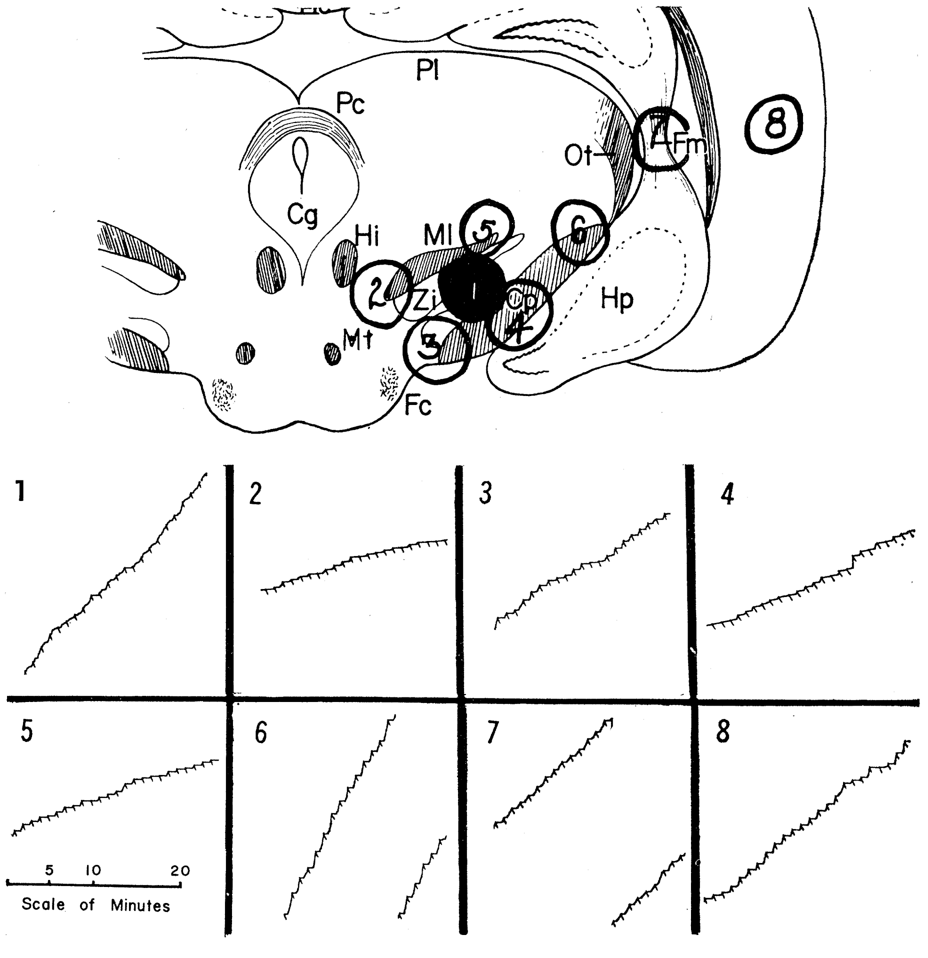

Fig. 2. Lesions placed along a theoretical path taken by the angled electrode to the mammillary body target. Lesion No. 1 (solid black) produced deficit in timing behavior. The other lesions, Nos. 2–8 (open circles) did not prevent appearance of fixed-interval scallop. Behavioral records 1–8 are results from lesions having the corresponding number in the diagram and are typical of the behavior on the ninth day of training. Abbreviations: Same as Fig. 1.

by the animals with the angled mammillary body lesion is likely due to the electrode track piercing fibers in this area.

The region of the zona incerta has intimate connections with the globus pallidus and extrapyramidal structures. In this connection Brady and Conrad (*4*) have already shown that when monkeys work for electrical stimulation of the globus pallidus on a reinforcement schedule where responses must be spaced 20 seconds apart, there is a marked shifting in the interresponse times towards the shorter time periods. This is fairly well localized, since electrical self-stimulation in other structures does not produce a comparable effect. These observations raise the possibility of the significance of extrapyramidal or basal ganglia structures in behaviors where temporal factors play a mediating role.

The observations presented in this report, together with our previously reported findings concerning the septum and hippocampus, de-emphasize the contribution of rhinencephalic structures to the temporal patterning of behavior. Moreover, it is suggested that the functional system principally involved in the temporal patterning of behavior appears to be extrapyramidal, as evidenced by the present results implicating the zona incerta, and the results of others implicating the globus pallidus (*5*).

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26 June 1963

Acetophenazine and Fighting Behavior in Mice

Abstract. *Assuming fear to be a variable of importance in the inhibition of aggressive behavior, we used acetophenazine to try to facilitate fighting in mature male C57BL mice. Drugged pairs did not differ significantly from controls in fighting latency; however, the time lapse from the first fight to submission and the actual fighting time to submission were significantly longer for drugged pairs. An attempt is made to relate what is known of the action of the drug with research on the neural correlates of aggressive behavior.*

A number of studies can be interpreted to support the hypothesis that fear is an important inhibitor of aggression. Hall and Klein (*1*) found that nonemotional rats were markedly more aggressive than an emotional strain. Seward (*2*) has emphasized "the conditioning of fear to stimuli associated with the fighting" in the reduction of aggression in male rats. King (*3*) observed long fighting-response latencies in mice raised in social isolation and concluded that they likely resulted from some inhibitory mechanism due to the strangeness of the social situation. Previous work by Knight (*4*) has indicated that rats which were raised in social isolation from weaning showed both physiological indications of stress and fought less when first caged together as adults.

Hess's success in extending the imprinting period by reducing fear with a phenothiazine (*5*) suggested to us that fighting behavior might also be facilitated by reducing the emotional response of laboratory mice with such a drug. Acetophenazine dimaleate (*6*) was selected because it was the most recently introduced phenothiazine derivative.

The phenothiazines depress the midbrain reticular formation, diminishing alertness. They also increase recruitment, that is, the sleep pattern mediated by the thalamocortical fibers, thus further diminishing alertness.

Both effects are additive and together tend to reduce emotional responses to stimuli. The phenothiazines depress the sympathetic function of the hypothalamus and also appear to block the action of the neurohormones, serotonin and noradrenaline (*7*). These drugs do not appear to affect the neocortex or the learning process (*8*). In our laboratory acetophenazine has been shown to reduce the rate of bar pressing for water by rats on a variable ratio schedule, without affecting acquisition (*9*).

It is hypothesized that if fear is an inhibitor of fighting, and if acetophenazine reduces fear without other significant effects upon behavior, acetophenazine is an agent which increases or prolongs fighting behavior.

Sixty-four male mice were used from the C57BL stock of Rockland Farms, New City, N.Y. Animals had been obtained at 8 weeks of age and caged in such a way that no subject could see another mouse.

The fighting cages consisted of a series of wire and wood-frame compartments about 30 by 15 by 15 cm. These compartments were arranged in a double row so that when a guillotine partition between two compartments was removed for the actual contest, each cage was about 61 by 15 cm. Food and water were available at all times.

When the mice were 10 to 12 weeks old, they were randomly paired and assigned to either the drug or the control treatment. On the fourth day after being placed into the contest cages, the mice were weighed and injected. Experimental pairs received 2.4 mg of acetophenazine per kilogram (*10*) and control pairs were injected with saline. All animals were injected subcutaneously with 0.1 ml of solution per 10 g of body weight.

One hour after injection the paired contests were undertaken and the following three measures recorded: (i) latency of the first fight; (ii) time lapse between the onset of the first fight and the first submissive posture of one member of the pair (latency to submission); and (iii) total time during which the mice were actually engaged in fighting before the first submission (duration of fighting). This final measure was not available on four of the total 160 pairings, owing to a failure in the timing device. A fight was said to have taken place when aggressive contact involving nipping occurred. Tail rattling and other threats were not recorded. It was assumed that both members of a pair were aggressive until a clear submission by one of the mice had occurred. The submissive posture is a stereotyped response which is easily recognizable and has been illustrated by Scott (*11*). Two observers were present for all contests, and in no instance did they disagree as to the response