Deficits in Passive Avoidance and Fear Conditioning in Mice with Septal Lesions

Abstract. *By means of a simple activity measure, mice with lesions of the septal forebrain were tested for passive avoidance (response inhibition) and fear conditioning. In two separate experiments animals with septal lesions showed little or no conditioning, as evidenced by lack of suppression of activity during and following activity-contingent foot shock. Results support and extend the hypothesis that these deficits in passive avoidance derive from the removal of normal inhibitory influences mediated by the septal area.*

While deficits in passive avoidance conditioning have been demonstrated in the rat (*1–3*) and cat (*4*) following lesions in the septal area, the mechanism underlying this behavioral loss is still not clear. Kaada, Rasmussen, and Kveim (*2*), McCleary (*4*), Schwartzbaum and Spieth (*3*), and others have suggested that these passive avoidance deficits derive from the removal of normal inhibitory influences provided by the septal area, resulting in a loss of response inhibition.

An alternative explanation is suggested by recent findings (*1, 5*) that show an increased intake of food and water after septal lesions. Thus the possibility exists that these passive avoidance deficits may derive simply from differences in drive level between experimental and control animals and are not necessarily related to removal of neural areas mediating response inhibition.

Indeed, Harvey *et al.* (*6*) demonstrated that deficits in inhibition of a conditioned response in rats with septal lesions were secondary to the increased thirst found in these animals after surgery. Since most previous demonstrations of passive avoidance deficits in animals with septal lesions utilized animals kept on a schedule of food or water deprivation, the results of Harvey *et al.* (*6*) raise a serious methodological issue with the response-inhibition hypothesis.

With simple activity measures, we found deficits in passive avoidance and fear conditioning in animals, with septal lesions, that were maintained with free access to food and water. Thus additional evidence is provided for the response-inhibition hypothesis.

In the two experiments reported, separate groups of experimentally naive, adult female mice, strain CF-1, were used. All animals were housed individually in commercial plastic or stainless steel cages and had food and water available at all times. In the first experiment, stereotaxically directed lesions were produced in the septal forebrain nuclei of 19 mice. Eight sham-operated and 22 normal mice served as controls. Training took place in a stainless steel box, measuring 25 by 18 by 16 cm, with a hinged lid. The floor was covered with four rectangular metal plates measuring 8 by 11 cm. Whenever an animal stepped from one plate to another it completed a circuit that allowed 20 μa of current to activate a transistor and, in turn, relay circuitry.

It could also be arranged that completion of the circuit between plates resulted in a shock for each animal of a 200-volt (d-c), 6-ma short-circuit current (*7*). Ten days after surgery each animal was tested for 2 minutes in the four-plate activity box. During the first minute (minute I) of the test free exploration was allowed; during minute II each activity response was automatically punished by a brief foot shock. Twenty-four hours later the activity of each animal was retested for 1 minute (minute III) with shock off. Suppression of activity from minute I to minute II yielded a measure of passive avoidance (response inhibition); suppression of activity from minute I to minute III yielded a measure of fear conditioning.

Results of this test are shown in Fig. 1. Kruskal-Wallis nonparametric analyses of variance for the activity scores among groups for each 1-minute test period were all significant ($P < .01$). Comparisons between individual groups showed no differences between normal and sham-operated animals. During the first minute of the test, animals with septal lesions were less active than controls. During minute II (shock-on) all control animals showed activity suppression (median decrease, 74 percent), while only 6 of the 19 mice with septal lesions showed activity suppression (*8*). As a group, animals with septal lesions actually showed a 26 percent increase in activity from minute I to minute II. When tested the next day (minute III), controls showed little activity (median decrease, 83 percent), while animals with septal lesions were still moderately active (median decrease, 25 percent).

In the second experiment the testing procedure was changed slightly in order to evaluate more closely the activity-contingent shock and in an attempt to produce greater suppression of activity in animals with septal lesions by lengthening the shock-on test period. Seven animals with septal lesions and seven normal animals were used. On the first test day each animal was placed in the four-plate box for 4 minutes. During the entire 4-minute period each response was punished by foot shock. Twenty-four hours later the animals were retested for 2 minutes with shock off. Activity was recorded every 15 seconds for each test period; results are shown in Fig. 2. Differences in total activity between groups for each test day are statistically significant ($P < .02$). In the shock-contingent situation animals with septal lesions not only take more shocks than controls do but also continue to make punished responses at a fairly steady rate throughout the 4-minute period. By the end of the first minute of the

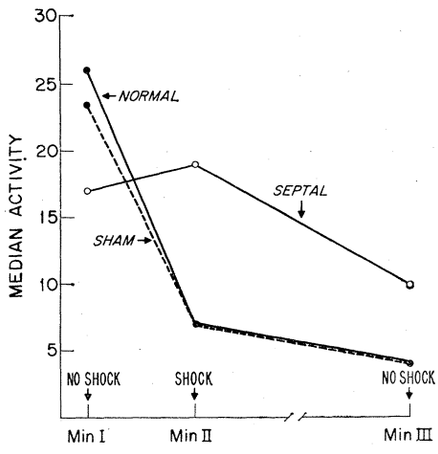

Fig. 1. Median activity for each 1-minute session of experiment 1.

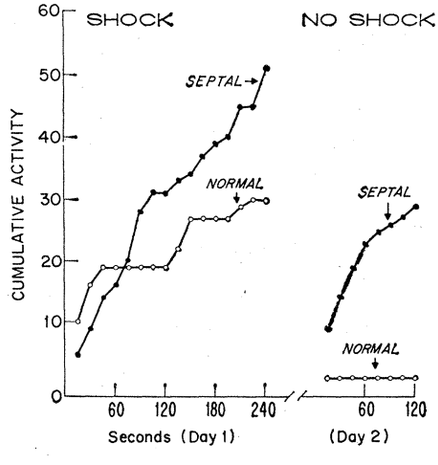

Fig. 2. Cumulative activity for each 15-second period of experiment 2.

test, control animals had emitted 63 percent of their total activity for the 4-minute period, while animals with septal lesions had emitted only 31 percent. When retested the next day, control animals showed almost complete suppression of activity (median decrease, 95 percent), while the activity of animals with lesions was virtually identical to that of the first 2 minutes of the test on the first day (median decrease, 3 percent).

Lesions of 13 of the 19 test animals in the first experiment and 5 of the 7 animals in the second experiment have been verified, either histologically or by inspection of free hand-cut sections. All lesions were placed within the septal area. Sizes of lesions varied from large and complete destruction of the septal area to small medially placed lesions that spared the lateral portion of the septum. All animals with lesions showed the syndrome of hyperexcitability previously described (*9*) although hyperexcitability was more pronounced in animals with the larger lesions. No obvious relation was seen between size of lesion and passive avoidance deficit. Several animals with virtually complete destruction of the septal area had scores overlapping those of controls, while other animals with destruction limited to the medial septal area showed little or no avoidance learning.

These results clearly demonstrate that lesions of the septal area in mice of strain CF-1 result in marked deficits of both passive avoidance and fear conditioning and that these deficits are not due simply to the motivation resulting from increased food or water intake in animals with septal lesions, as indicated by previous reports.

That the deficits in our study do not stem from hyperactivity in animals with septal lesions is demonstrated by test minute I in the first experiment in which these animals were found to be less active than controls. Nor is the septal deficit simply a result of a burst of responding following the first shocked responses. In the second experiment animals with septal lesions actually took fewer shocks than controls in the first minute of the test but continued to make punished responses over the 4-minute test period. In the last 15-second period of this test 71 percent of the animals with septal lesions, but only 29 percent of the controls, were still moving.

The fact that animals with septal lesions appear less able to inhibit activity-contingent shock and show little or no evidence of activity suppression when replaced in the experimental chamber the following day strongly suggests that the lesions interfere with some central inhibitory mechanism. While our results also concur with the hypothesis that septal lesions reduce the punishing effect of shock (*10*) or reduce fear (*9*), recent studies in this laboratory have shown that mice with septal lesions, as well as controls, acquire a fear response in a test situation that does not require activity suppression (*11*).

The behavioral response underlying activity suppression in the present experiment is probably "freezing," a response that is dominant in cats, rats, and mice in a fear-arousing situation. Interference with this mode of responding would appear to account for the results of this experiment.

BURTON M. SLOTNICK
Department of Psychology, Columbia University, New York
MURRAY E. JARVIK
Department of Pharmacology, Albert Einstein College of Medicine, New York

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Sensitivity of Cardiac Actomyosin to Calcium

Katz and Repke (*1*) have recently reported that reconstituted actomyosin from dog heart muscle has a calcium sensitivity similar to that from skeletal muscle and that half maximum activities of adenosine triphosphate hydrolysis for heart and skeletal muscle actomyosin occur at a *p*Ca of 6.20 and 6.25, respectively.

It should be pointed out that the *p*Ca at half maximum activity calculated in this case from the EGTA/CaEGTA ratio is of the same order of magnitude as that calculated with the use of the ATP/CaATP ratio from the previously reported data on the effect of Ca on cardiac natural actomyosin (*2*). Half maximum adenosine triphosphatase activity for natural heart actomyosin was obtained at a total calcium concentration of about $10^{-5}M$. If we take the association constants for the reactions, $H^+ + ATP^{-4} \leftrightharpoons ATP^{-3}$, $Mg + ATP^{-4} \leftrightharpoons MgATP^{-2}$, and $Ca + ATP^{-4} \leftrightharpoons CaATP^{-2}$, as 10^7, 8×10^4, and 3×10^4, respectively (*3*), the calculated *p*Ca is 5.87. There is some uncertainty in the values of the association constants. If, for instance, the association constant for $CaATP^{-2}$ is taken to be the same as that for $MgATP^{-2}$, that is, 8×10^4, *p*Ca becomes 6.25. Undoubtedly the same uncertainty attaches to the calculation of *p*Ca from the EGTA-CaEGTA equilibrium which involves the four protonation equilibrium constants of EGTA and the corresponding association constants for the Ca complexes.

Syneresis of cardiac myofibrils has been reported (*4*) to be totally inhibited even with an added calcium concentration of $5 \times 10^{-6}M$, but there is no inhibition at a total calcium concentration of $3 \times 10^{-5}M$. This concentration is somewhat higher than that required for skeletal myofibrils under the same (*4*) or similar (*5*) experimental conditions but could probably be accounted for by other calcium-binding contaminants present with heart myofibrils (*6*).

These data support the general concept that only a very small concentration of free Ca^{++}, of the order of 10^{-6} to $10^{-7}M$, is necessary for Mg-activated adenosine triphosphatase activity of the cardiac as well as the skeletal muscle actomyosin system.

BARRY L. FANBURG
New England Medical Center Hospital, Boston, Massachusetts

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