Cholinergic Involvement in the Behavioral Effects of Septal Lesions

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Septal lesions differentially affect the performance of rats in a variety of aversive learning situations (see Caplan, 1973; Lubar, 1973; Defrance, 1976, for reviews). Many studies, for example, have demonstrated that septally-lesioned animals are deficient in passive avoidance learning (see Fried, 1972). Likewise, septal lesions severely disrupt the performance of rats in leverpress shock escape tasks (Gotsick, Osborne, Allen, & Hines, 1971). In contrast, septal lesions facilitate the acquisition of 2-way active avoidance and rats with septal lesions perform more efficiently than normal rats on Sidman avoidance tasks (Morgan & Mitchell, 1969). While several explanations have been proposed to explain these apparently discrepant results (see Caplan, 1973; Fried, 1972; Lubar, 1973), the most parsimonious explanation appears to be that septal lesions reduce freezing responses in aversive situations (Blatt, 1976; Mattingly, Osborne & Gotsick, 1979).

Following septal lesions there is a significant reduction in the levels of several forebrain neurotransmitters including serotonin, acetylcholine, and the catecholamines, dopamine and norepinephrine (see Defrance, 1976). Recently, it has been suggested that the behavioral changes following septal damage are a consequence of the lesion-induced reduction of brain serotonin. Supporting evidence for this view is as follows: (a) Animals treated with para-chlorophenylalanine (PCPA), a compound which inhibits the synthesis of serotonin and thereby depletes brain serotonin, are similar to septally-lesioned rats in some aversive learning tasks. For example, PCPA-treated rats, like septal-lesioned rats, are deficient in passive avoidance but are superior to normal rats in active avoidance (see Peters, Anisman, & Papas, 1978); and (b)
the facilitation of active avoidance learning following septal lesions is reversed by the administration of 5-hydroxytryptophan (5-HTP), a compound which increases brain serotonin levels (Smith, 1979).

Although serotonin does seem to be involved in the septal lesion-induced changes in avoidance learning, recent experiments in our laboratory indicate that a reduction in brain serotonin cannot explain all the behavioral effects of septal lesions in aversive learning situations. As examples, we have found that rats treated with PCPA are less active than normal rats during an aversive conditioned stimulus (CS) (Mattingly, Chandler, Applegate, & Brunelle, 1983), whereas septally-lesioned rats are more active than normal rats during an aversive CS (Mattingly et al., 1979). Further, although septally-lesioned rats are deficient in leverpress shock escape learning (Gotsick et al., 1971), rats treated with PCPA learn to escape shock as quickly as control rats (Mattingly, Graham & Applegate, 1981). Finally, the deficient shock escape performance of rats with septal lesions is not improved by the administration of 5-hydroxytryptophan (Mattingly, Gotsick & Applegate, 1982). It is evident from these results, therefore, that the reduction of brain serotonin consequent to septal damage is not responsible for all of the observed lesion-induced behavioral changes.

As mentioned, septal lesions produce a reduction in the levels of other neurotransmitters besides serotonin. It is possible, therefore, that some of the behavioral changes observed following septal damage are a result of these other neurochemical changes. Over the past two years we have been studying the possible involvement of dopamine and acetylcholine in the effect of septal lesions. Although dopaminergic systems do not appear to be involved, we do have suggestive evidence for the involvement of acetylcholine. Specifically, we have found that scopolamine, a central acetylcholine receptor blocker, produces
a number of behavioral changes in a leverpress shock escape task which are very similar to those produced by septal lesions (Mattingly, 1983). This indirect evidence, of course, suggests that lesion-induced decreases in brain acetylcholine may be responsible for some of the behavioral effects of septal lesions.

The objective of the proposed research, therefore, was to obtain more direct evidence linking reduced brain acetylcholine with the effects of septal lesions. Specifically, the present experiment determined whether the effects of septal lesions on shock escape learning in rats could be reversed by a drug (pilocarpine) which facilitates acetylcholine activity. If the deficient shock escape learning of rats with septal lesions is a result of reduced acetylcholine activity, then increasing acetylcholine activity should attenuate this deficit.

Method

Subjects and Design. Forty Wistar albino rats, experimentally naive, and approximately 90 days old were used as subjects. The rats were housed individually, maintained on ad lib food and water and a 12 hour light-dark cycle was held constant throughout the experiment. The rats were randomly assigned, in equal numbers, to four groups comprising a 2 x 2 factorial design combining a lesion factor (Septal vs. no lesion) and a drug factor (pilocarpine vs. saline). Specifically, approximately 14 days prior to behavioral testing, one group of rats (N=20) was given septal lesions and the rest of the rats were given sham-operations. Then one-half of the rats in both the lesion and no-lesion group were given an injection of pilocarpine or saline 30 min prior to behavioral testing.
Surgical Procedures. All surgery was performed using ether anesthesia. Bilateral lesions of the septum were produced through a stereotaxically oriented electrode connected to a Radionics radio frequency lesion maker (Model RFG-4). The electrode tip was positioned 1.5 mm anterior to Bregma, 1.0 mm lateral to the midline at an angle of 5°, and 5.0 mm below dura. Current was passed for 20 seconds and the tip temperature reached between 60-70°C. Rats in the control group were anesthetized and placed in the stereotaxic instrument. The scalp was incised, but the skull was not penetrated. At the conclusion of behavioral testing, all lesioned animals were sacrificed using ether anesthesia and perfused intracardially with physiological saline followed by a 10% formalin solution. The brains were then removed, and after sufficient fixation in 10% formalin, frozen coronal sections were cut, mounted on slides, and examined microscopically to determine the extent of damage to the septum.

Pharmacological Procedures. Doses (1.0 mg/kg) of pilocarpine hydrochloride were calculated as the active base of the drug and dissolved in physiological saline daily prior to administration. All injections were given intraperitoneally (IP). Control animals received an injection of an equivalent volume of saline. Also, treatment conditions were coded so that group assignments were unknown to the experimenter during both injection and testing procedures.

Apparatus and Procedure. Behavioral testing was conducted in two identical Grason-Stadler experimental rat chambers (Model 1111) housed in sound attenuated research chests. These chambers have a grid floor and a response lever mounted on one wall. Grason-Stadler constant current shock generators (Model 700) equipped with grid scramblers were used to administer footshock. In each training session, an animal was placed in one of the
chambers and 90 sec later, a 1 mA footshock was delivered. This shock continued until the rat pressed the lever or until 1 min elapsed. The next shock trial began following the 90 sec intertrial interval. Each subject was tested in a single session consisting of 60 discrete shock trials. During the session, response latencies to the nearest .001 sec were recorded. In addition, the total number of leverpresses per session and the total amount of time during the session the response lever was depressed was recorded. All behavioral contingencies were programmed and controlled by a TRS-80 Model III computer connected to the behavioral testing equipment via a Lafayette computer interface system.

Results

Behavioral

Figure 1 presents the mean speed scores across blocks of 10 trials for septally-lesioned and control rats treated with either pilocarpine (Pilol) or saline (Sal) prior to testing. Speed scores were derived by adding the integer one to each latency and then taking the reciprocal (1/\(1/LAT+1\)). This transformation prevents very short or very long latency scores from making a disproportionate contribution to the mean performance score. The possible range of transformed scores is from zero to one, with larger numerical values representing faster response speeds.

As may be seen in Figure 1, speed scores increased for all groups of rats across trial blocks. Further, rats injected with pilocarpine appeared to respond more slowly than rats treated with saline. This difference in response speed between saline- and pilocarpine- treated rats, however, appeared to be greater for the control rats than for lesioned rats. Finally, lesioned rats responded as
Figure 1. Mean speed scores across blocks of 10 trials for septally-lesioned and control rats treated with either pilocarpine (PILO) or saline (SAL) prior to testing.
quickly control rats when treated with saline, but lesioned rats appeared to respond more quickly than control rats when treated with pilocarpine.

These data were analyzed using a three-factor mixed analysis of variance with lesion and drug as between factors and trial-blocks as a within factor. The results of this analysis indicated that neither drug nor lesion were significant factors affecting response speed. Indeed, the only significant effect revealed by this analysis was the block effect; $F(5,170) = 35.63, p < .001$.

**Anatomical**

Examination of brain sections revealed a relatively small amount of damage to the septum in all lesioned animals. Moreover, this damage in most animals was restricted to that lateral septal nuclei leaving the medial septal nucleus intact in nearly all animals.

**Discussion**

In previous studies (Gotsick et al., 1971; Mattingly et al., 1982), septally-lesioned rats were found to be severely deficient, relative to normal rats, in lever-press shock escape learning. In the present study, however, the performance of rats with septal lesions did not differ significantly from control rats. This discrepancy between the results of the present and previous studies seems most likely attributable to differences in the extent of damage to the septal nuclei. In previous studies reporting behavioral deficits, both the medial and lateral nuclei of the septum were destroyed bilaterally (Gotsick et al., 1971; Mattingly et al., 1982). Although a similar amount of damage was intended in the present study, the actual amount of damage to the septum was very small and was restricted largely to the lateral septal nuclei. Thus, it would appear that damage to the medial septal nucleus is critical in producing this
particular behavioral deficit. The main objective of the present study was to test the hypothesis that the deficient shock escape performance of rats with septal lesions is a result of the lesion-induced decrease in brain acetylcholine levels. Specifically, the purpose of this study was to determine whether injections of the cholinergic against, pilocarpine, would significantly improve the retarded shock escape performance of rats with septal lesions. Unfortunately, the very small lesions produced and the lack of a behavioral deficit in lesioned rats injected with saline prevented this determination from being made. The results do, however, provide some suggestive evidence that pilocarpine may have had the predicted effect if the lesions had included the medial septal nuclei. For example, although the performance of lesioned and control rats injected with saline was virtually identical, septally-lesioned rats injected with pilocarpine tended to respond more quickly than control rats injected with pilocarpine. Hence, it seems likely that if the lesions had been larger, pilocarpine would have had the predicted effect. Moreover, the lack of a significant behavioral deficit in septally-lesioned rats treated with saline may also indirectly support this hypothesis. That is, the most recent neurohistochemical evidence indicates that the majority of acetylcholine-containing neurons in the septum are located in the medial septal nuclei and project to the hippocampus (Lewis & Shute, 1978). Since the medial septal nuclei were not significantly damaged in the present study, the level of brain acetylcholine was probably not significantly reduced. Consequently, a lesion-induced behavioral deficit would not have been predicted by the "reduced-acetylcholine" hypothesis of effects of septal lesions.
References

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