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Scopolamine Disrupts Leverpress Shock Escape Learning in Rats

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MATTINGLY, B. A. *Scopolamine disrupts leverpress shock escape learning in rats.* PHARMACOL BIOCHEM BEHAV 24(6) 1635–1638, 1986.—Five groups of rats were tested on a discrete-trial leverpress shock escape task 30 min following an intraperitoneal injection of either 0 (saline), 0.2, 1.0, or 5.0 mg/kg scopolamine hydrobromide, or 5.0 mg/kg methylscopolamine hydrobromide. The results indicated that scopolamine, but not methylscopolamine, disrupted escape performance and this disruption was dose-related. These findings are consistent with both disinhibition and reduced freezing explanations of anticholinergic effects and support the view that reduced acetylcholine is involved in the behavioral effects of septal lesions.

Rats Shock escape Scopolamine Cholinergic mechanisms Acetylcholine Septal lesions

SEPTAL lesions differentially affect the performance of rats in a variety of aversive learning situations (see [6, 8, 14] for reviews). Many studies, for example, have demonstrated that septally-lesioned animals are deficient in passive avoidance learning [11]. Likewise, septal lesions severely disrupt the performance of rats in leverpress shock escape tasks [13]. 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 [19]. While several explanations have been proposed to explain these apparently discrepant results [6, 11, 14], the most parsimonious explanation appears to be that septal lesions reduce freezing responses in aversive situations [4,16].

Following septal lesions there is a significant reduction in the level of several forebrain neurotransmitters (see [12,27]). Recently it has been suggested that the behavioral changes observed in aversive learning tasks following septal damage are a consequence of the lesion-induced reduction of brain serotonin [25–26]. 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 [23]; 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 [26].

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) [15], whereas septally-lesioned rats are more active than normal rats during an aversive CS [16]. Further, although septally-lesioned rats are deficient in leverpress shock escape learning [13], rats treated with PCPA learn to escape shock as quickly as control rats [17]. Moreover, the deficient shock escape performance of rats with septal lesions is not improved by the administration of 5-hydroxytryptophan [17]. It is evident from these results, therefore, that the reduction of brain serotonin consequent to septal damage is not solely responsible for all of the observed lesion-induced behavioral changes in aversive learning tasks.

Besides serotonin, septal lesions produce a reduction in the levels of other neurotransmitters. Indeed, acetylcholine levels are significantly reduced following septal lesions [22, 24, 27], and many similarities have been reported between the behavioral effects of septal lesions and cholinergic blockade (see [2–3] for reviews). Moreover, in some nonaversive situations a pharmacological dissociation has been demonstrated in the behavioral effects of septal lesions. For example, septal lesions have been reported to disrupt the habituation of both exploratory and startle responses, whereas cholinergic blockade via scopolamine, disrupts the habituation of exploratory, but not startle, responses [29]. In contrast, depletion of brain serotonin via para-chlorophenylalanine, disrupts the habituation of startle, but not exploratory, responses [7]. Since septal lesions result in reductions of both acetylcholine and serotonin, these habituation findings suggest that the various behavioral effects of this lesion may be related to one or the other neurochemical change.

As mentioned, septal lesions produce a significant disruption in leverpress shock escape performance [13], but the performance of rats on this task is not affected by manipulations of brain serotonin levels. The purpose of the present study, therefore, was to determine whether the performance of rats on this task would be affected by a drug-induced interference with central cholinergic functioning. Consequently, groups of rats were injected with either saline (con-

trol) or the central cholinergic antagonist, scopolamine (0.2, 1.0, or 5.0 mg/kg) and then tested on a leverpress shock escape task. In addition, another group of rats was injected with 5.0 mg/kg of methylscopolamine, a peripheral cholinergic antagonist, prior to testing to control for peripheral effects of scopolamine. If reduced brain acetylcholine is responsible for the behavioral effects of septal lesions on this task, then rats treated with scopolamine, like septally lesioned rats, should be deficient in shock escape learning and should show other behavioral characteristics of rats with septal lesions.

METHOD

Subjects

Fifty male Wistar albino rats were experimentally naive and approximately 90 days old on the day of testing. All rats were housed individually and maintained on ad lib food and water. A 12 hour light-dark cycle was held constant throughout the experiment.

Apparatus

Behavioral testing was conducted in two Grason-Stadler operant conditioning chambers (Model IIII) housed individually in sound attenuated research chests. These chambers had grid floors, and a house light (GE 1820) and response lever mounted on one wall. Grason-Stadler constant current shock generators (Model 700) equipped with grid scramblers were used to deliver footshock.

Design and Procedure

Prior to the initiation of testing, the rats were randomly assigned, in equal numbers, to five drug condition groups. Three groups were injected intraperitoneally (IP) with doses of 0.2, 1.0, or 5.0 mg/kg of scopolamine hydrobromide. One group was injected with saline, and the final group was injected IP with a 5.0 mg/kg dose of methylscopolamine hydrobromide. All injections were given 30 min before the shock escape training session. All doses were calculated as the active base of the drug and dissolved in isotonic saline just prior to administration. Also, all doses were administered in a volume of 1 ml/kg and treatment conditions were coded so that group assignments were unknown to the experimenter during injection and testing procedures. Following the injection all rats were returned to their home cage.

Shock escape training was initiated 30 minutes following the drug injection. In the test session, the rat was placed into one of the chambers and 90 sec later a 1.0 mA footshock was delivered to the grid floor. This shock continued for 1 min or until the rat pressed the lever. The shock trials were separated by 90 sec intervals and the test session consisted of 60 discrete shock escape trials. During the test session, response latencies to the nearest 0.001 sec were recorded. These response latencies were converted to speed scores by adding the integer one to each latency (in sec) and then taking the reciprocal (i.e., 1/(latency + 1)). This transformation prevents very short or very long latencies scores from making a disproportionate contribution to the mean performance scores. The possible range of transformed scores is from zero to one, with larger numerical scores representing faster speeds [21]. Besides response latencies, the total number of leverpresses (Barpresses) and the total amount of time the lever was depressed (Bartime) during the session was also recorded.

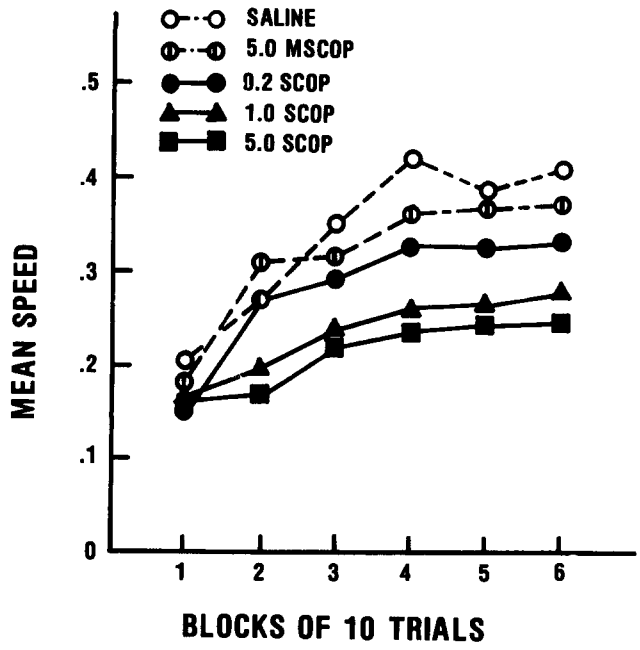

FIG. 1. Mean speed scores (1/(latency +1)) for the five groups across blocks of 10 escape trials.

RESULTS

Figure 1 presents the mean speed scores for the groups across 6 blocks of 10 shock escape trials. As may be seen in Fig. 1, all groups displayed an increase in speed scores across blocks, but the saline and methylscopolamine groups responded more quickly than the scopolamine-injected groups. An analysis of variance performed in these data revealed a significant effect of blocks, $F(5,225)=37.86$, $p<0.001$, and a significant drug treatment effect, $F(4,45)=4.71$, $p<0.01$. A Neuman-Keuls post hoc analysis performed on the significant drug effect indicated that the saline-injected rats responded faster than the 0.2, 1.0, and 5.0 mg/kg scopolamine-injected rats, $p<0.05$ in each case. Further, the methylscopolamine-injected rats responded significantly faster than the 1.0 and 5.0 mg/kg scopolamine injected rats, $p<0.05$ in each case. However, the saline group did not significantly differ from the methylscopolamine group in speed of responding, $p>0.05$.

Table 1 presents the mean barpresses, bartime, and bartime per barpress for the five drug groups. An analysis of variance performed on each of these measures revealed a significant drug effect: barpresses, $F(4,45)=3.79$, $p<0.01$; bartime, $F(4,45)=12.35$, $p<0.0001$; and bartime per barpress (BTBP), $F(4,45)=10.85$, $p<0.0001$. As may be seen in Table 1, the number of barpresses and amount of bartime was markedly decreased by scopolamine. More important, the decrease in bartime was not simply a function of decreased barpressing. Indeed, the saline-treated rats held the lever down an average of 7.0 sec for each barpress, whereas the rats injected with 5.0 mg/kg of scopolamine averaged less than 0.3 sec of bartime per barpress.

DISCUSSION

It is evident from these results that scopolamine disrupts leverpress shock escape performance of rats in a dose-related fashion. In contrast, the escape performance of rats

TABLE 1

SUMMARY OF MEAN BARPRESSES, BARTIME AND BARTIME/BARPRESS (BT/BP) FOR THE FIVE DRUG GROUPS

Drug	Barpress	Bartime	BT/BP
Saline	191.2^{A}	1258.2^{A}	7.0^{A}
Methylscop	111.2^{B}	527.3^{B}	4.9^{A}
Scop 0.2 mg/kg	128.7^{B}	95.7^{C}	0.5^{B}
Scop 1.0 mg/kg	124.6^{B}	46.4^{C,D}	0.3^{B}
Scop 5.0 mg/kg	100.6^{B}	27.1^{D}	0.3^{B}

Means with different letter superscripts are significantly different as determined by Neuman-Keuls tests, $p<0.05$.

treated with methylscopolamine was not significantly different from that of the saline-control rats. Thus, the disruption in escape learning produced by scopolamine would appear to be due primarily to the central, rather than peripheral, effects of the drug.

Previous studies of leverpress shock escape learning (e.g., [13,17]), have shown that "normal" rats learn to escape shock quickly by staying near the lever and holding it down during the intertrial intervals. Consequently, escape speed is usually directly related to the amount of time the lever is depressed (bartime). Rats with septal lesions, however, have difficulty remaining near the lever during the intertrial intervals, and therefore, display very little bartime and much slower escape latencies than do normal rats. This pattern of slow escape responding and decreased bartime in rats with septal lesions is, of course, very similar to that observed in the present study with scopolamine-injected rats. Since septal lesions produce a significant reduction in acetylcholine activity, the results of the present study are consistent with the view that reduced brain acetylcholine may be responsible for the lesion-induced retardation of leverpress shock escape performance.

The reduced bartime and slow escape responding in rats with septal lesions and scopolamine-treated rats may be consistent with several explanations of cholinergic and septal function. However, two particular hypotheses seem most relevant. The first view suggests that a septal-hippocampal cholinergic system mediates response inhibition (e.g., [9,10]). According to this view, septal and scopolamine-treated rats are deficient in their ability to withhold or inhibit competing responses. Thus, in a leverpress shock escape task both groups of rats, unlike normal rats, are unable to inhibit competing exploratory responses during the intertrial intervals and therefore, do not remain near the lever during the intertrial intervals. Thus, these rats display little bartime and little improvement in escape speed. A similar, but more limited, explanation of these findings suggests that septal lesions and scopolamine treatments simply reduce the tendency of rats to freeze in aversive or fear producing situations (e.g., [1, 16, 18]). Freezing responses, as well as flight responses in aversive situations are considered to be "species-specific defense reactions" (SSDRs). Bolles [5] has stated that avoidance and escape responses are acquired by the elimination or suppression of ineffective SSDRs. On active avoidance tasks, for example, the freezing response must be suppressed before optimal performance is obtained. Conversely, in passive avoidance tasks, freezing responses lead to effective performance, whereas the flight reaction must be suppressed. Similarly, in a leverpress shock escape task, a tendency to freeze on the lever during the intertrial interval would facilitate the escape response. According to this view, then, the disruption of leverpress shock escape performance in rats with septal lesions and those treated with scopolamine is related to a reduced tendency to freeze in aversive situations.

It should be noted that while the present results are consistent with inhibition and freezing explanations of cholinergic function, other more recent theories of cholinergic functioning which emphasize the role of acetylcholine in acquisition and retention processes, particularly with regard to spatial navigation (see [20,28] for review), cannot be excluded. Unfortunately, leverpress shock escape tasks, like many simple tasks used in psychopharmacological research, cannot differentiate among inhibitory, memory, or discrimination processes when used alone (see [30]). Consequently, it cannot be concluded from such tests that cholinergic antagonists affect only inhibitory or freezing processes. Likewise, it is of course possible that the deficient shock escape performance of the scopolamine-treated rats may have been related to a direct effect on sensory or motor systems independent of higher level cognitively inferred processes. For example, scopolamine has been reported to increase both shock sensitivity and locomotor activity (see [3]). Either of these two reported effects of scopolamine could be involved in the scopolamine-induced disruption of leverpress shock escape performance. Para-chlorophenylalanine, however, has also been reported to increase shock sensitivity and locomotor activity (see [23,25]), but this drug *does not* disrupt the shock escape performance of rats [17]. Hence, drug-induced changes in shock sensitivity and/or locomotor activity alone would not appear to account for the disruption in shock escape performance.

Regardless of the behavioral mechanisms responsible for the present findings, the similarity between the effects of scopolamine treatments and septal lesions on shock escape performance does support the view that a lesion-induced reduction in brain acetylcholine may be responsible for some of the behavioral effects of septal lesions. It should be noted, however, that septal lesions and anticholinergics do not always have the same behavioral effects [2], and consequently, reduced brain acetylcholine probably plays an important, but not an exclusive, role in determining the effects of septal damage.

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REFERENCES

1. Anisman, H. and T. G. Waller. Effects of inescapable shock on subsequent avoidance performance: Role of response-repertoire changes. *Behav Biol* **9:** 331–355, 1973.
2. Bignami, G. Nonassociative explanations of behavioral changes induced by central cholinergic drugs. *Acta Neurobiol Exp* **36:** 5–90, 1976.
3. Bignami, G. and H. Michalek. Cholinergic mechanisms and aversively motivated behaviors. In: *Psychopharmacology of Aversively Motivated Behavior*, edited by H. Anisman and G. Bignami. New York: Plenum Press, 1978.
4. Blatt, R. C. Facilitation and nonfacilitation of active avoidance behavior of rats with septal lesions in the shuttle box and running wheel. *J Comp Physiol Psychol* **90:** 704–714, 1976.
5. Bolles, R. C. Species-specific defense reaction. In: *Aversive Conditioning and Learning*, edited by F. R. Brush. New York: Academic Press, 1971.
6. Caplan, M. An analysis of the effects of septal lesions on negatively reinforced behavior. *Behav Biol* **9:** 129–167, 1973.
7. Carlton, P. L. and C. Advokat. Attenuated habituation due to parachlorophenylalanine. *Pharmacol Biochem Behav* **1:** 657–663, 1973.
8. DeFrance, J. R. (Editor). *The Septal Nuclei*. New York: Plenum Press, 1976.
9. Douglas, R. J. Pavlovian conditioning and the brain. In: *Inhibition and Learning*, edited by R. Boakes and M. Halliday. London: Academic Press, 1972.
10. Douglas, R. J. The development of hippocampal function: Implications for theory and therapy. In: *The Hippocampus: Vol 2: Neurophysiology and Behavior*, edited by R. C. Isaacson and K. H. Pribram. New York: Plenum Press, 1975.
11. Fried, P. A. Septum and behavior: A review. *Psychol Bull* **78:** 292–310, 1972.
12. Gage, F. H., R. G. Thompson and J. J. Valdes. Endogenous norepinephrine and serotonin within the hippocampal formation during the development and recovery from septal hyperactivity. *Pharmacol Biochem Behav* **9:** 359–367, 1978.
13. Gotsick, J. E., F. H. Osborne, C. J. Allen and K. M. Hines. Factors affecting performance on a shock escape task in rats with septal lesions. *Physiol Behav* **6:** 199–201, 1971.
14. Lubar, J. F. and R. Numan. Behavioral and physiological studies of septal function and related medial cortical structures. *Behav Biol* **8:** 1–25, 1973.
15. Mattingly, B. A., H. Chandler, E. B. Applegate and M. Brunelle. Activity during a conditioned aversive stimulus in rats treated with para-chlorophenylalanine. *Psychol Rec* **34:** 389–396, 1984.
16. Mattingly, B. A., F. H. Osborne and J. E. Gotsick. Activity changes during a conditioned aversive stimulus in rats with septal lesions. *Physiol Behav* **22:** 521–525, 1979.
17. Mattingly, B. A., J. E. Gotsick and E. B. Applegate. Serotonin, septal lesions, and shock escape learning in rats. *Psychol Rep* **59:** 3–9, 1986.
18. Mollenauer, S., R. Plotnik and P. Southwick. Scopolamine: Effects on fear or defense responses in the rat. *Pharmacol Biochem Behav* **5:** 157–163, 1976.
19. Morgan, J. M. and J. C. Mitchell. Septal lesions enhance delay of responding on a free operant avoidance schedule. *Psychon Sci* **16:** 10–11, 1969.
20. Murray, C. L. and H. C. Fibiger. Pilocarpine and physostigmine attenuate spatial memory impairments produced by lesions of the nucleus basalis magnocellularis. *Behav Neurosci* **100:** 23–32, 1986.
21. Osborne, F. H. Varied shock and simple escape. *Psychol Rep* **29:** 1231–1236, 1971.
22. Pepeu, G., A. Mulas, A. Ruffi and P. Sotgiu. Brain acetylcholine levels in rats with septal lesions. *Life Sci* **10:** 181–184, 1971.
23. Peters, D. A. V., H. Anisman and B. A. Pappas. Monoamines and aversively motivated behavior. In: *Psychopharmacology of Aversively Motivated Behavior*, edited by H. Anisman and G. Bignami. New York: Plenum Press, 1978.
24. Sethy, V. H., M. J. Kuhar, R. A. Roth, M. H. VanWoert and G. K. Aghajanian. Cholinergic neurons: Effect of acute septal lesion on acetylcholine and choline content of rat hippocampus. *Brain Res* **55:** 481–484, 1973.
25. Smith, R. F. Mediation of footshock sensitivity by serotonergic projection to hippocampus. *Pharmacol Biochem Behav* **10:** 381–388, 1979.
26. Smith, R. F. Attenuation of septal lesion-induced shuttlebox facilitation by 5-hydroxytryptophan. *Physiol Psychol* **7:** 419–421, 1979.
27. Sorensen, J. P. and J. A. Harvey. Decreased brain acetylcholine after septal lesions in rats: Correlation with thirst. *Physiol Behav* **6:** 723–725, 1971.
28. Whishaw, I. Q. Cholinergic receptor blockade in the rat impairs locale but not taxon strategies for place navigation in a swimming pool. *Behav Neurosci* **99:** 979–1005, 1985.
29. Williams, J. M., L. W. Hamilton and P. C. Carlton. Pharmacological and anatomical dissociation of two types of habituation. *J Comp Physiol Psychol* **87:** 724–732, 1974.
30. Zolman, J. F., B. A. Mattingly and C. L. Sahley. Cholinergic involvement in inhibitory behavior of the young domestic chick. *Behav Biol* **23:** 415–432, 1978.