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BRIEF COMMUNICATION

Effects of 3-Acetylpyridine on Spontaneous Alternation in the Mouse

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BLAMPIED, N. M. AND C. M. WILBY. *Effects of 3-acetylpyridine on spontaneous alternation in the mouse.* PHARMAC. BIOCHEM. BEHAV. 3(2) 317–319, 1975. –When administered to mice, 3-acetylpyridine has been shown to selectively destroy the hippocampal neural fields CA_3 and CA_4. Adult mice, injected i.p. with 150 mg/kg 3-acetylpyridine showed a reduced frequency of spontaneous alternation (48%) in a T-maze, compared with saline injected controls (73%). The pattern of latency change in the experimental mice was consistent with a failure to habituate normally. These behavioral effects of 3-acetylpyridine resemble those observed following lesions of the hippocampus induced by stereotaxic surgery.

Hippocampus Chemical lesions 3-Acetylpyridine Antimetabolites Spontaneous alternation Habituation T-maze Mice

PRODUCING central nervous system lesions by systemically injecting a substance which is selectively taken up at a specific neural locus, with subsequent degeneration of the vulnerable tissue, is a technique for inducing lesions which combines simplicity of administration with a high degree of precision of effect. One such substance is gold-thioglucose, which produces medial hypothalmic lesions in the mouse, thereby inducing obesity [12, 14, 16]. Another is 3-acetylpyridine (3-AP), an antimetabolite of the vitamin nicotinamide [23]. Hicks [8] reported that when 3-AP was administered to mice, in doses of fatal magnitude, it produced a pattern of lesions involving the adrenal medulla, supra-optic nucleus, and the pyramidal layer of the hippocampus. Coggeshall and MacLean [2] employing an LD_{50} dose of 300–350 mg/kg in mice, found that pathology was evident within hours of the injection, and was confined to the areas CA_3 and CA_4 of the hippocampus. Subsequent work showed that this pattern of hippocampal damage was found in all strains of mice tested, but it was not reliably observed in the rat, guinea pig or cat [15]. In the squirrel monkey, 3-AP was found to damage the lateral geniculate and the inferior olive ([3], MacLean, personal communication, 1973).

No systematic examination of the behavioural effects of 3-AP seems to have been reported, although several investigators have suggested its use as an adjunct to traditional techniques in the localization of functions within the hippocampus [2,21]. As a test of the effects of 3-AP on behavior, the present experiment used spontaneous alternation, a behavior which has been shown to be particularly sensitive to hippocampal distruption [5, 6, 7]. An emergence test was also included as a test of emotionality [9].

METHOD

Animals

Twelve adult mice, NZ mixed strain (the supplier was unable to specify the strain more exactly), 6 male, 6 female, were used. They were housed in groups segregated by sex, and maintained on a reversed day–night cycle, with ad lib food and water.

Apparatus

The emergence test employed a modified home cage with metal walls 20 cm long, 19 cm wide, with a hinged wire mesh lid. The floor was built up with paper strips and wood shavings to within approximately 4 cm of the top of the cage.

Spontaneous alternation was studied in a floorless T-

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maze, 10 cm high, 10 cm wide and 33 cm long, with a hinged perspex lid. One side was painted white, the other black. The startbox (10 X 10 X 7.5 cm) was separated from the stem by a sliding aluminium partition, and aluminium barriers could be inserted either side of the choice point. The maze was placed approximately 1 m beneath a 22 W light on a paper floor, and the paper was changed after each individual animal had completed its trials. White masking noise was supplied to the experimental room at all times.

Procedure

Drug administration. Animals were randomly assigned to form an experimental and a control group, balanced for sex. The 6 mice in the experimental group were injected i.p., with 150 mg/kg 3-AP divided into two doses each of 75 mg/kg, given 24 hr apart. The 3-AP was supplied (Sigma Chemicals) in liquid form and was injected undilute. The 6 control mice were injected with an equal volume of isotonic saline, also in 2 divided doses. Forty-eight hr elapsed from the final injection to the beginning of testing.

Emergence test. Each animal was placed in the test cage and confined for 30 sec. The lid was then raised and the latency of the emergence of the whole head above the wall of the cage was recorded with a stopwatch.

Spontaneous alternation. Ten pairs of trials were given, one pair per day, in the T-maze. On each trial the animal was confined in the startbox for 5 sec, then the door was raised and the latency to leave the startbox was recorded. A four-footed entry into one of the arms completed the trials, and the animal was confined for 30 sec in the chosen arm before being returned either to the startbox for the second trial, or to its home cage. A trial was terminated, and the animal removed if it failed to make a choice within 10 min of entering the maze.

RESULTS

None of the experimental mice showed any of the ill-effects of 3-acetylpyridine reported by Coggeshall and MacLean [2].

In the emergence test the mean emergence latency was 7.3 sec for the control mice, and 7.4 sec for the experimental mice, a non-significant difference ($t < 1.0$).

The maximum possible alternation score in the spontaneous alternation task was 10. The mean score for the control group was 7.3 with a range of 6 to 9, and the mean score for the experimental group was 4.8 with a range of 2 to 6; a significant difference ($t = 4.4$, $df = 10$, $p < 0.01$).

The T-maze latency data were analysed using a Groups X Trials X Days analysis of variance ([22], p. 337). The two groups did not differ significantly on latency scores, but Trial 2 latencies were significantly greater than Trial 1 scores, $F(1,10) = 8.68$, $p < 0.05$, and latencies increased significantly over days, $F(9,90) = 4.01$, $p < 0.05$. As Fig. 1 shows, the control animals exhibited a substantial increase in Trial 1 latencies and an even greater increase in Trial 2 latencies, over the 10 days. The experimental group showed a negligible increase Trial 1 latencies, and a greater, but still relatively small increase in Trial 2 latencies, over days. These differences in the amount by which Trial 1 and Trial 2 latencies increased over days, and between the groups, account for the significant Groups X Days and Trials X Days interactions, $F(9,90) = 3.32$ and 2.12, respectively, $p < 0.05$.

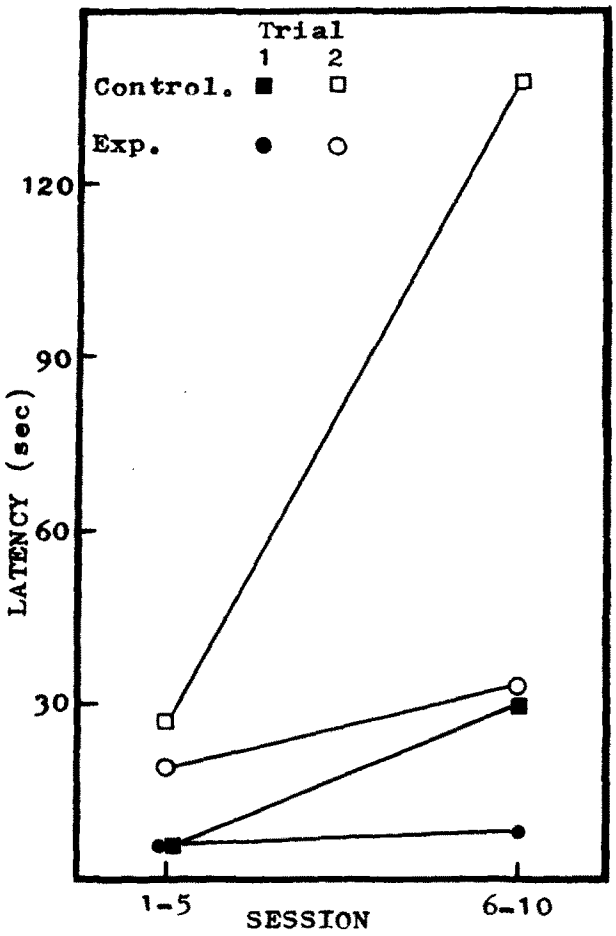

FIG. 1. Mean changes in first and second trial latencies for control and experimental groups, averaged over the first 5 and last 5 sessions.

DISCUSSION

The control group animals alternated with a mean frequency of 73%, close to the value of 74% reported by Petchkovsky and Kirby [18] for normal mice. Spontaneous alternation was reduced to chance levels in the mice given 3-AP; the mean alternation rate for this group was 48%. This effect of 3-AP resembles the effect of large hippocampal lesions in rats which also abolish spontaneous alternation [5,19]. No comparison data exist for mice stereotaxically lesioned in the hippocampus.

The lower rate of alternation in the experimental mice cannot be attributed to differences in emotionality, as measured by the emergence test, nor were the groups different, overall, in latency to leave the startbox. However, there were differences between the groups as measured by the latency data. Normal mice increase their startbox latency over successive trials [18], and the control mice showed a similar pattern in this study. Mice given 3-AP continued to emerge from the startbox on Trial 1 with little increase in latencies over the 10 days. On the second trial each day their latencies increased although not to the same extent as did the controls. Comparison latency data from animals with hippocampal lesions are lacking, but the

observed pattern of latency change is similar to the pattern of change in running speed reported by Leaton [13]. He observed that control animals decreased their running speed over trials, while rats lesioned in the hippocampus continued to run on each trial at the same speed. This failure to increase latency over trials is consistent with a failure to habituate normally with repeated exposure to the same environment, and may more generally reflect an impairment in memory processes. A reduced rate of habituation is typically observed in rats with hippocampal lesions [10, 11, 20].

In conclusion, the behavior of mice administered 3-AP is consistent with the hippocampal neuropathology this substance is known to produce. If these results are confirmed or extended, they add a further element to the issue of the functional heterogeneity of the hippocampus [4, 7, 17] since spontaneous alternation is differentially affected by dorsal and ventral stereotaxic lesions [21], and has been related to both the rate and extent of cell maturation in the dentate gyrus, by comparison between species known to mature at different rates [6] and by X-irradiation interference with post-natal cell development [1].

REFERENCES

1. Bayer, S. A., R. L. Brunner, R. Hine and J. Altman. Behavioral effects of interference with the postnatal acquisition of hippocampal granule cells. *Nature New Biol.* **242:** 222–224, 1973.
2. Coggeshall, R. E. and P. D. MacLean. Hippocampal lesions following administration of 3-Acetylpyridine. *Proc. Soc. exp. Biol. Med.* **98:** 687–689, 1958.
3. Coggeshall, R. E. and P. D. MacLean. Cerebral effects of 3-Acetylpyridine in the squirrel monkey (*Samiri sciureus*). *Trans. Am. neural Ass.* **85:** 169–170, 1959.
4. Douglas, R. J. The hippocampus and behavior. *Psychol. Bull.* **67:** 416–442, 1967.
5. Douglas, R. J. and R. L. Isaacson. Hippocampal lesions and activity. *Psychon. Sci.* **1:** 187–188, 1964.
6. Douglas, R. J., J. J. Peterson and D. P. Douglas. The ontogeny of a hippocampus-dependent response in two rodent species. *Behav. Biol.* **8:** 27–37, 1973.
7. Ellen, P. and J. Deloache. Hippocampal lesions and spontaneous alternation behavior in the rat. *Physiol. Behav.* **3:** 857–860, 1968.
8. Hicks, S. P. Pathologic effects of antimetabolites: I. Acute lesions in the hypothalamus, peripheral ganglia and adrenal medulla caused by 3-Acetylpyridine and prevented by nicotinamide. *Am. J. Path.* **31:** 189–199, 1955.
9. Hughes, R. N. Effects of early postweaning handling on the development of exploration in the rat. *Aust. Psychologist* **5:** 324, 1970. (Abstract)
10. Jarrard, L. E. and B. N. Bunnel. Open field behavior of hippocampal lesioned rats and hamsters. *J. comp. physiol. Psychol.* **66:** 500–502, 1968.
11. Kamback, M. Effect of hippocampal lesions and food deprivation on response for stimulus change. *J. comp. physiol. Psychol.* **63:** 231–235, 1967.
12. Larsson, S. and L. Strom. Some characteristics of goldthioglucose obesity in the mouse. *Acta physiol. scand.* **38:** 298–308, 1957.
13. Leaton, R. N. Exploratory behavior in rats with hippocampal lesions. *J. comp. physiol. Psychol.* **59:** 325–330, 1965.
14. Liebelt, R. A. and J. H. Perry. Hypothalamic lesions associated with goldthioglucose-induced obesity. *Proc. Soc. exp. Biol. Med.* **95:** 774, 1957.
15. MacLean, P. D. Comments on the selective vulnerability of the hippocampus. In: *Selective Vulnerability of the Brain in Hypoxaemia*, edited by J. P. Schade and W. H. McMenemey. London: Blackwell, 1963, pp. 177–181.
16. Marshall, N. B., R. J. Barnett and J. Mayer. Hypothalamic lesions in goldthioglucose injected mice. *Proc. Soc. exp. Biol. Med.* **90:** 240, 1955.
17. Nadel, L. Dorsal and ventral hippocampal lesions and behavior. *Physiol. Behav.* **3:** 891–900, 1968.
18. Petchkovsky, L. and B. J. Kirkby. Individual differences, emotionality and spontaneous alternation in mice. *Aust. J. Psychol.* **22:** 75–77, 1970.
19. Roberts, W. W., W. N. Dember and M. Brodwick. Alternation and exploration in rats with hippocampal lesions. *J. comp. physiol. Psychol.* **55:** 697–700, 1962.
20. Sengstake, C. B. Habituation and activity patterns of rats with large hippocampal lesions under various drive conditions. *J. comp. physiol. Psychol.* **65:** 504–506, 1968.
21. Stevens, R. and A. Cowey. Effects of dorsal and ventral hippocampal lesions on spontaneous alternation, learned alternation and probability learning in rats. *Brain Res.* **52:** 203–224, 1973.
22. Weiner, B. J. *Statistical Principles in Experimental Design.* New York: McGraw-Hill, 1962.
23. Woolley, D. W. *The Biochemical Bases of Psychoses.* New York: Wiley, 1962.