Pharmacology Biochemistry & Behavior, Vol. 10, pp. 369–372. Printed in the U.S.A.

PA-Learning in Young Rats With Dorsal Hippocampal- and Hippocampo-Entorhinal Atropine

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(Received 2 July 1978)

BLOZOVSKI, D. *PA-learning in young rats with dorsal hippocampal- and hippocampo-entorhinal atropine*. PHARMAC. BIOCHEM. BEHAV. **10**(3) 369–372, 1979.—Twenty-one-day-old rats injected with atropine into dorsal hippocampus and trained on a white-black step-through passive avoidance task, did not perform differently from their controls in acquisition or extinction. In contrast, when atropine was administered into the ventral hippocampo-entorhinal area, the animals displayed a passive avoidance deficit. The results support the finding that the posteroventral but not the anterodorsal part of the hippocampal complex is implicated in passive avoidance learning and suggest a cholinergic mediation of this effect.

Atropine Hippocampal complex Passive avoidance learning Young rat

A NUMBER of experiments have demonstrated that extended hippocampal damage can interfere with passive avoidance learning [14, 15, 16, 18, 21, 26, 31]. Furthermore, other studies involving small lesions have suggested that the anterodorsal and posteroventral parts of the hippocampal complex are functionally distinct in modulating various behaviors, especially passive avoidance.

Discrete lesions restricted to the posterior part of the hippocampus proper have been shown to interfere with the acquisition and retention of passive avoidance [19], whereas no significant effect was seen after damage to its anterodorsal part [19,24]. It has also been reported that large lesions to the entorhinal cortex (some of them involving incidental encroachments upon the caudal CA1 subsector, the ventral subiculum, and the posterior presubiculum) can induce passive avoidance deficits [9, 28, 34].

The hippocampus has also been proposed as an important part of central cholinergic pathways mediating response suppression in nonreward and punishment situations [6, 7, 8, 17]. Because the systemic blockade of the muscarinic cholinergic transmission impairs passive avoidance learning, at least in some experimental conditions [3, 4, 5, 8, 23,35], it might be possible that anticholinergic compounds injected directly into dorsal or ventral loci of the hippocampal complex would differentially affect passive avoidance learning.

A recent study [27] has, however, failed to demonstrate any disturbing effect on a punished ingestive passive avoidance task following administration of antimuscarinic or antinicotinic agents in the dorsal or ventral hippocampus proper. Because the ventral site in that study was relatively dorsal, the present experiments examined the effects of injections into a more ventral locus in the hippocampal complex, one involving the most ventral part of the hippocampus proper with adjacent subiculum and entorhinal cortex.

Because the systemic administration of anticholinergics impairs passive avoidance not only in adult but also in young rats [1, 11, 37], and because it was demonstrated in our previous work [1] that the rate of acquisition and the disturbing effects of atropine injected intraperitoneally reach the adult levels after the 21st day of life, the present investigation was undertaken at 21 days in order to determine whether the same task would be affected by atropine injected either into the anterodorsal hippocampal formation or into the posteroventral hippocampo-subiculo-entorhinal area.

METHOD

Animals

Forty 21-day-old male and female rats of the London Black strain *(Rattus norvegicus)* weighing 39–51 g were used in the experiment; they were divided into two groups, one receiving bilateral injections in the anterodorsal hippocampal formation (DH), the other in the posteroventral hippocampo-subiculo-entorhinal area (VHE). Within each group, 10 experimental animals were injected with atropine sulfate (ATR) and 10 animals used as controls received an equivalent volume of saline solution (NaCl). The animals were bred in our laboratory, each litter being limited to 8 pups on Day 1 or 2 after birth. The animals lived with their mother until the day of experiment. On that day, two animals of the same sex and approximately equal weight were chosen in each litter, one being randomly assigned to the experimental subgroup and the other to the control one.

Surgical Procedure

Surgery was carried out in two 3-min stages 60–90 min apart, both under light ether anesthesia, using a stereotaxic

apparatus as modified by Nadler [25]. The first operation consisted in the preparation of the skull (skin incision and cranial perforation). Recovery from anesthesia was very rapid. During the second operation, a 300-μm needle was lowered into the desired sites, the solution injected, and the needle pulled out. Behavioral recovery was complete 10–12 min after injection. Bilateral coordinates of the injection sites were 2 mm posterior to bregma, 1.8 mm lateral to the midline, and 2.8 mm below the cranial surface for the DH group, and 2.5 mm anterior to lambda, 4.5 mm lateral to the midline, and 6.8 mm below the cranial surface for the VHE group.

Drug Administration

The atropine sulfate solution in saline was freshly prepared on the day of experiment. Fifty μg of atropine were administered on each side in a volume of 0.8 μl delivered over a 30-sec interval in experimental animals, and 0.8 μl of the saline solution was injected in controls. All injections took place 16–20 min before the beginning of the acquisition session. In a preliminary study, drug diffusion was measured by adding Niagara Sky Blue to the solution, and was estimated to conform to a sphere about 1.3 mm in dia.

Apparatus and Procedure

The apparatus was identical to that previously described [1]. Briefly, it consisted of a double-compartment chamber, one compartment white, the other black, provided with a grid floor. Each animal, when placed on the white side, moved rapidly into the black compartment; as soon as all 4 paws were on the grid floor, a partition between both sides was closed, a constant current shock 0.2 mA, 50 Hz, 0.5 sec duration was delivered, and the rat immediately removed to a holding cage for 3 min. Each animal had up to 10 trials to learn to remain for 300 sec in the white compartment. Cross-through latencies were recorded and a 300-sec score was assigned to all trials subsequent to the terminal acquisition trial. An extinction procedure of 10 trials maximum followed immediately acquisition, each trial consisting of a 30-sec exposure to the black side without shock, followed by a retest for passive avoidance in the white compartment. The criterion for extinction was the crossing into the black compartment within 300 sec.

Histology

At the end of each experiment, the animal was killed with an overdose of ethyl carbamate, the brain was frozen and the site of injection histologically verified. Some of the brains were fixed in Bouin's solution and prepared for histological examination using Bodian's method [2].

Statistical Analysis

Drug effects and differences between groups were evaluated nonparametrically by means of the two-tailed Wilcoxon t-test for paired samples, and the two-tailed Mann-Whitney U-test for unpaired samples. The criterion for statistical significance was $p \leq 0.05$ (two-tailed).

RESULTS

Figure 1 represents a composite diagram showing all of the cannula tip locations for experimental and control animals in both the DH and VHE groups. All the dorsal hippocampal injections were placed in the dentate gyrus and some of them also extended to the CA hippocampal layers. The majority of the ventral placements invaded both the ventral CA layers of the hippocampus near the subiculum and the adjacent entorhinal areas.

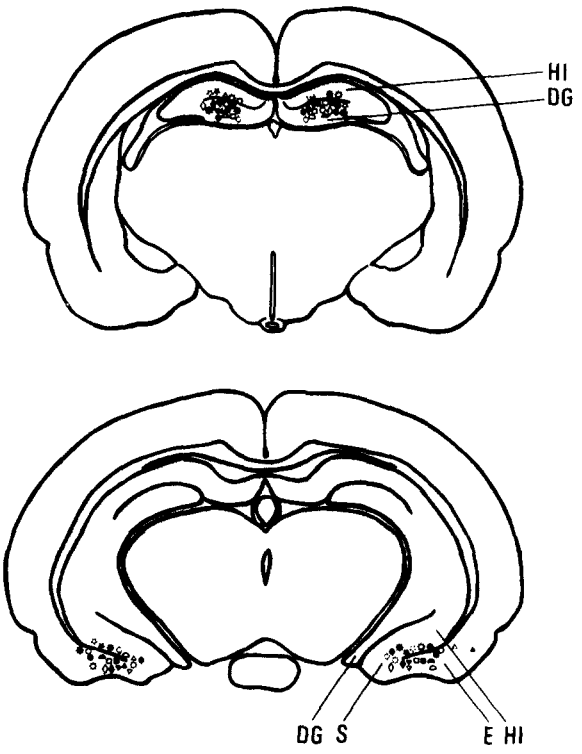

FIG. 1. Composite diagram showing all cannula tip locations for atropine- (black symbols) and control (open symbols) animals in groups DH (top) and VHE (bottom). DG=dentate gyrus, E=entorhinal area, HI=hippocampus, S=subiculum.

Figure 2 summarizes the performances of the DH group (A) and the VHE group (B), during acquistion and extinction. Individual comparisons for the total cross-through latencies between experimental and control animals within the DH group (by means of the Wilcoxon t-test) revealed no differences during acquistion ($t=16$, $N=10$, $p>0.05$) nor during extinction ($t=5$, $N=10$–4 ex aequo, $p>0.05$), indicating that the (ATR-DH)-animals performed as well as the (NaCl-DH)-rats during both acquisition and extinction. Similar comparisons within the VHE group indicated that the (ATR-VHE)-animals showed slower acquisition ($t=0$, $N=10$, $p<0.01$) and faster extinction ($t=0$, $N=10$–1 ex aequo, $p<0.01$) as compared to the (NaCl-VHE)-animals.

Moreover, comparisons for the total cross-through latencies between the control groups (NaCl-DH) and (NaCl-VHE) by means of the Mann-Whitney U-test revealed no differences in acquisition ($U=39$, $N_1=N_2=10$, $p>0.10$) or in extinction ($U=38$, $N_1=N_2=10$, $p>0.10$), indicating that acquisition and resistance to extinction had similar courses following injections of the vehicle solution in either locus. Similar comparisons between the experimental groups (ATR-DH) and (ATR-VHE) revealed significant differences in acquisition ($U=16$, $N_1=N_2=10$, $p<0.02$) and extinction ($U=14$, $N_1=N_2=10$, $p<0.02$), thus demonstrating, as was

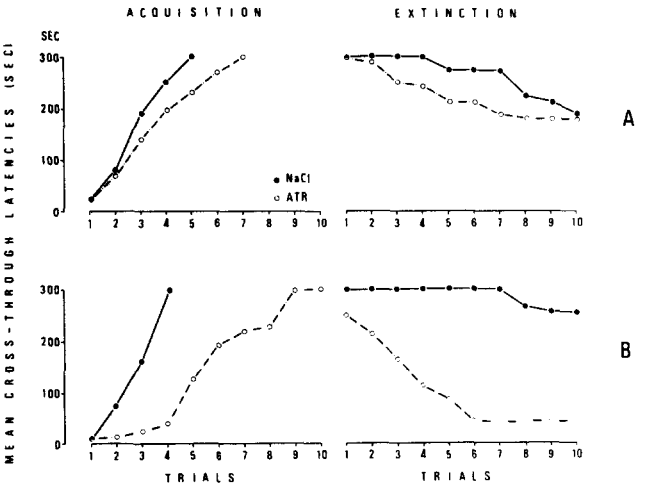

FIG. 2. Acquisition (left) and extinction (right) of passive avoidance learning in 21-day-old rats following bilateral injections of atropine (ATR) and saline (NaCl) into DH (A) and VHE (B) regions measured by the mean cross-through latencies as a function of trials.

shown by the Wilcoxon analyses, that atropine has differential effects when injected in those loci.

DISCUSSION

The results of the present experiments indicate that, in 21-day-old rats at least, learning of a passive avoidance task is not affected by atropine administered to the anterodorsal hippocampal formation, whereas it is impaired by drug injections into the posteroventral part of the hippocampo-subiculo-entorhinal area. The present observation provides additional support for the conclusion that the dorsal and ventral portions of the hippocampal complex are functionally separate entities, the later being important for passive avoidance behavior [19, 24, 33]. It is also in agreement with the finding that substantial deficits in passive avoidance are obtained after lesions of the entorhinal cortex [9, 28, 34], subiculum and cingulum [12]. Because the ventral hippocampus, subiculum and entorhinal cortex are anatomically connected [13,36], it should not be surprising if they were also functionally related for the mediation of passive avoidance responses: in fact, it has been proposed that the entorhinal area and hippocampus interact so as to exercise common influences on behavior, especially on passive avoidance [9,28].

Our results suggest that muscarinic cholinergic pathways are implicated in the mediation of passive avoidance. In fact, a cholinergic transmission should be possible in the VHE region since AChE staining was evidenced in zone "31" of the hippocampo-subicular area, in layers I and III of the presubiculum [22,30], and in layer IV of the entorhinal areas [13]. Furthermore, a major afferent pathway, the cingulum, projects from the medial frontal cortex and cingulate area to the dentate gyrus, hippocampus, presubiculum, retrosplenial area, parasubiculum and entorhinal areas, especially in layer IV [36]. In addition, the cingulate cortex has been shown to contain cholinoceptive neurons [20]. Thus, the hippocampal complex might have, in addition to the well known major cholinergic pathway originating rostrally in the septum [22,32], another cholinergic input originating in the frontal and cingulate cortex and projecting caudally in the posteroventral part of the hippocampo-subiculo-entorhinal area. The fact that a cholinergic involvement was not shown for passive avoidance when atropine was injected in the ventral hippocampus in an earlier report [27] may be due to the fact that the injection did not involve the extreme ventral hippocampo-subiculo-entorhinal area.

In the present work, the (ATR–VHE)-animals exhibited slower acquisition and faster extinction when compared to the (NaCl–VHE)-rats. Similar disturbing effects have been shown to occur following intraperitoneal administration (IP) of atropine to rats of the same age studied in an identical learning situation [1]. However, the acceleration of extinction manifested in the present group (ATR–VHE) was less marked than that shown in [1] by (ATR–IP)-animals, probably because there was a slight resistance to extinction in group (NaCl–VHE) as compared to group (NaCl–IP). Because it was demonstrated in adult rats that ventral hippocampal lesions produce a slower rate of extinction of a passive avoidance response without affecting acquisition [24], it seems probable that the performance of the (ATR–VHE)-animals studied here reflects some interaction of drug and cannula-induced damage.

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