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Time-Dependent Retention Deficits Induced by Post-Training Injections of Atropine into the Caudate Nucleus[1]

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PRADO-ALCALÁ, R. A., L. SIGNORET AND M. FIGUEROA. *Time-dependent retention deficits induced by post-training injections of atropine into the caudate nucleus*. PHARMAC. BIOCHEM. BEHAV. 15(4) 633–636, 1981.—Rats were trained on a one-trial passive avoidance task, and retention of the task was measured 24 hr later. Atropine was injected bilaterally into the anterior caudate nuclei (ACN) of rats from independent groups at one of several intervals after training. Application of atropine 2 min after training produced a lack of retention of passive avoidance. An intermediate degree of impairment was seen when the treatment was given 3 min 45 sec after the learning experience, and interference with retention was still noted when an interval of 7 min 30 sec was studied. In contrast, no deficits were observed in groups of animals injected with atropine 15 or 30 min after training. Rats injected with atropine into the parietal cortex 2 min after training showed only a minimal reduction of retention, and a group injected with saline solution into the ACN performed as well as non-treated animals. These results suggest that there is a time-dependent process that mediates the retention of passive avoidance, and that this process requires the activation of cholinergic synpases within the anterior caudate nucleus.

Caudate nucleus Memory Cholinergic activity Passive avoidance Cholinergic blockade Atropine Learning Retention

AN increasing number of experimental findings lend strong support to the hypothesis that the functional integrity of the anterior caudate nucleus (ACN) is necessary for the development of instrumentally conditioned behaviors, mediated by both positive [2] or negative [14] reinforcers. Further, there is reason to think that these types of behaviors are mediated by cholinergic activity of the ACN: amnesia is induced when anticholinergic drugs are applied to the ACN before testing the capacity of animals to perform a conditioned response [7,12] as well as when these agents are administered shortly after a learning experience [5,11]. In contrast, improvement in learned performance is seen when acetylcholine (ACh) [9] or its precursor choline [4,9] are injected into the ACN.

It has been reported that post-training electrical stimulation of the caudate impairs the retention of a passive avoidance task [16,17]. It has also been demonstrated that the induction of retrograde amnesia is time-dependent, i.e., amnesia was produced by treatments administered shortly, but not long, after training [15].

To our knowledge, no attempts have been made to define the temporal limits of the effects of cholinergic blockade of the ACN on memory processes. The present experiment was based on the findings reported above [15] and on the idea that cholinergic activity of the caudate nucleus plays a critical role in learning processes [3, 5, 11]. It was predicted that an inverse relationship would be found between the time of atropine injection into the ACN after training in a passive avoidance task and the degree of subsequent impairment of retention of that training.

METHOD

Animals

Sixty-two experimentally naive male Wistar rats, weighing between 250–350 g were used. They were individually lodged and had free access to solid food (Purina Laboratory Chow) and tap water. Under Nembutal anesthesia (40 mg/kg), 46 animals were submitted to bilateral implantation of double-walled cannulae made with hypodermic needle tubing (outer, 21 gauge; inner, 27 gauge) aimed at the dorsal aspect of the anterior caudate nucleus (A-P=bregma, L=3.0 mm, H=−3.5 mm from the dural surface); in 8 rats cannulae were similarly implanted in the parietal cortex, using the same coordinates as those for the caudate placements, ex-

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cept for the dorsal-ventral depth (H = −0.5 mm). Stereotaxic coordinates were obtained from the König and Klippel atlas [6]. In all cases bregma and lambda were positioned in the same horizontal plane, perpendicular to the cannulae shaft. The animals were allowed one week to recover from surgical procedures before training was begun. The remaining 8 rats served as unoperated controls.

Apparatus

Training and testing were conducted in a box with two symmetrical compartments, separated by means of a guillotine-type door (Lafayette Instr). Each compartment measured 30 cm × 20 cm × 20 cm, and the grid floor of one of the compartments (stainless steel bars, 0.5 cm in diameter, separated 2.0 cm center-to-center) could be electrified by means of a Grass S-44 stimulator that was connected in series with stimulus isolation and constant current units. To distinguish the shock compartment from the opposite (safe) compartment, the walls of the latter had vertical strips of dark-colored tape and the floor was wire mesh.

Training and Testing

On the only training session each rat was placed inside the safe compartment and after 10 sec the guillotine door was opened. The time from the opening of the door until the rat moved with all four paws into the shock compartment was recorded. As soon as the rat entered the shock compartment, the door was closed and footshock was applied for 5 sec (square-wave pulses of 60 msec and 1.0 mA at 100 ppsec). When these 5 sec had elapsed, the door was reopened and the shock was continued until the rat had escaped to the safe compartment. Twenty-four hr later the same procedure was followed, except that footshock was not administered to any of the rats (test of retention). If during the retention test a rat failed to move into the shock compartment within 600 sec, its trial was terminated and a score of 600 was assigned.

Groups

Animals with cannulae implanted in the ACN were randomly assigned to one of the following groups: those to be injected with 60 μg of atropine through each cannulae at 2 min (CN-2′, n=8), 3 min 45 sec (CN-3′45″, n=8), 7 min 30 sec (CN-7′30″, n=6), 15 min (CN-15′, n=6) or 30 min (CN-30′, n=8) after training and a group to be injected bilaterally with saline 2 min after training (CN-0, n=10). The group of animals with cannulae in the parietal cortex were designated as Ctx-2′ (n=8) and were to be administered 60 μg of atropine bilaterally 2 min after training. And, as stated above, there was a control group of 8 unimplanted rats (UI).

Treatments

In a previous experiment in which the same passive avoidance task was studied [4], we found a dose-dependent impairment of retention performance when atropine was injected bilaterally into the ACN 6 min post-training. In that 60 μg per injection was the minimum dose that produced maximal retention deficits, this dose was chosen for the present study.

Sixty μg of atropine sulphate (Merck), dissolved in 3 μl of a saline solution(final osmolarity, 250 mOsM; pH 5.9) were injected through each cannula of all implanted animals, except in those of the 0 dose group (CN-0) in which 3 μl of 0.9% NaCl (217 mOsM; pH 5.8) were injected bilaterally. The dose of atropine refers to the salt. The bilateral injections were performed outside the training-testing room and were administered through 27 gauge delivery cannulae at the rate of 1 μl/20 sec. In each group, half the animals were injected first in the right hemisphere and then in the left hemisphere; for the remaining half this sequence was reversed. The delivery cannulae were left inside the guide cannulae for an additional min after each injection.

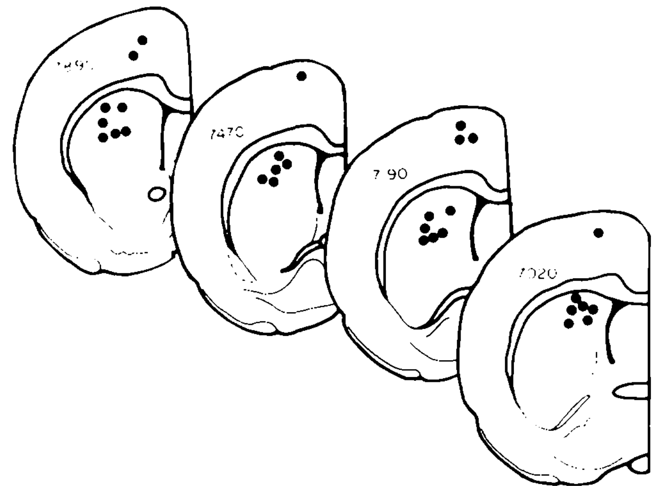

FIG. 1. Schematic representation of cannulae placements in the caudate nucleus and in the parietal cortex (all placements are represented in the right hemisphere). The number of placements shown is smaller than the total number of implanted cannulae because different animals had cannulae tips in the same location. The coronal sections are in the plane of the König and Klippel atlas [6].

Statistics

Kruskal-Wallis ANOVA was computed on latencies to cross to the shock compartment during both training and testing sessions. When appropriate, Mann-Whitney U tests were performed between pairs of groups.

Histology

When behavioral testing was concluded, all cannulated rats were deeply anesthetized and perfused intracardially with isotonic saline followed by 10% Formalin. Their brains were removed and stored in 10% Formalin for at least two weeks before frozen coronal sections (40 μm thick) were stained using the Nissl method to aid in determination of location of cannulae tips.

RESULTS

Histological analysis demonstrated that, as depicted in Fig. 1, the tips of all cannulae that were implanted in the caudate nucleus were located between A-P 7.0 and 8.0 in the dorsal half of the anterior aspect of this structure. The tips of the cortical cannulae were within the A-P limits mentioned above [6].

As expected from results of similar experiments [4,11], there were no significant differences among the groups with regard to latencies to cross into the shock compartment during the training session, nor in total time of footshock.

TABLE 1

RETENTION SESSION

Group	UI	Ctx-2′	CN-0	CN-2′	CN-3′45″	CN-7′30″	CN-15′	CN-30′
n	8	8	10	8	8	6	6	8
Mean	558.0	403.8	412.0	18.9	303.4	486.3	499.0	527.0
S.D.	21.4	169.5	239.0	34.3	224.7	131.7	165.2	146.2
Md	558.5	461.0	549.5	7.0	310.5	535.5	508.0	580.0
Ctx-2′	9.5‡							
CN-0	27.5, NS	22.0, NS						
CN-2′	0.0§	0.0§	1.0§					
CN-3′45″	14.5*	20.0, NS	18.0*	3.0§				
CN-7′30″	10.0*	18.0, NS	17.0, NS	0.0§	13.0, NS			
CN-15′	24.0, NS	13.0, NS	20.0, NS	0.0§	9.0*	11.0, NS		
CN-30′	19.5, NS	9.0‡	17.0†	0.0§	10.0‡	9.0*	17.0, NS	

Comparisons of retention scores between every pair of groups, on the retention test (Mann-Whitney U tests, one-tailed p values).

Abbreviations as follows: n, sample size; Mean, mean retention scores; S.D., standard deviations; Md, median. Groups: UI, Unimplanted rats; Ctx, injected with atropine (60 μg) into each parietal cortex. The rest of the groups were bilaterally injected with 60 μg of atropine into the caudate nucleus at the following intervals after training: 2 min, 3 min 45 sec, 7 min 30 sec, 15 min or 30 min (CN-2′, CN-3′45″, CN-7′30″, CN-15′ and CN-30′, respectively); group CN-0 was injected with saline solution 2 min after training.

The figures in each column represent the computed U values. NS, non-significant differences; p values are represented by *, 0.05; †, 0.025; ‡, 0.01; §, 0.001.

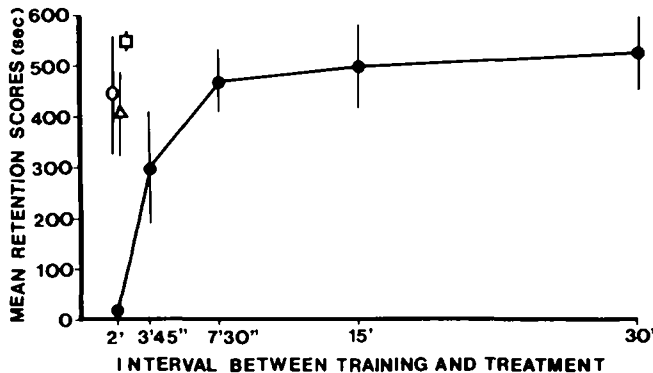

FIG. 2. Mean retention scores (ordinate) are plotted against intervals between training and treatment application (abscissa). Rats were injected with 60 μg of atropine (solid circles) or with saline solution (open circle) into the caudate nucleus. Also shown are retention scores of a group that was bilaterally injected with 60 μg of atropine into the parietal cortex (triangle), and of a group of nontreated rats (square). Standard deviations are also represented for each group.

Hence, it can be stated that all groups of animals, regardless of cannulae implantation, had the same response capabilities and that any observed differences in retention were due to the effects of the treatments. This outcome allowed us to use a scoring system that is commonly used in studies of passive avoidance. The retention score for each animal was computed by subtracting the latency to cross during the retention test from the latency to cross during training. Larger scores indicate better retention whereas lower scores reflect less retention of the passive avoidance training.

Comparison of retention scores among all groups revealed a highly significant treatment effect, H=29.25, df=7, p=0.0005, as seen in Fig. 2. As shown in Table 1, subsequent U tests showed that retention scores of the unimplanted group of rats were not significantly different from those of the CN-0, CN-15′ or CN-30′ groups but were significantly higher than the scores of each of the rest of the groups. On the other hand, the group of rats that was injected with atropine into the ACN two min after training (CN-2′), had a significantly lower retention score than any of the other groups. A reliable deficit was also displayed by the CN-3′45″ group as its score was significantly lower than the scores of the UI, CN-0, CN-15′ and CN-30′ groups. Finally, the performance of group CN-7′30″ was only significantly lower than that of UI and CN-30″ groups.

DISCUSSION

This is the first report that demonstrates that administration of an anticholinergic drug to the caudate nucleus induces a time-dependent impairment in the processes that underlie retention of an instrumental task. Atropine injections into the ACN, delivered two min after training, produced a complete lack of passive avoidance when animals were tested 24 hr later. An intermediate degree of impairment was seen when the treatment was applied 3 min 45 sec after the learning experience, and a slight interference with retention was still noted when an interval of 7 min 30 sec was studied. On the other hand, no deficits could be observed in the groups of animals that were given the anticholinergic agent 15 or 30 min after training.

These results enable us to postulate that the mechanisms of retention of passive avoidance require the activation of

cholinergic synapses in the caudate nucleus. Thus, when cholinergic blockade of the ACN is produced shortly after training, the process underlying retention is prevented from occurring. With intermediate intervals this process is stopped before complete learning is achieved, and with intervals of 15 min or greater optimal retention can be observed because the engram for passive avoidance has been fully developed.

Interestingly enough, other workers have found a temporal gradient for retention that is very similar to the one described in the present experiment. Wyers and Deadwyler [15] studied the effects of electrical stimulation of the ACN on a passive avoidance task; they found that stimulation at 0.5, 2.0 or 5.0 min after training produced a marked retention deficit, and there were no detrimental effects when the current was delivered beyond 15 min.

The observation that amnesia is induced by the application of atropine into the ACN is consistent with previous reports in which the same dose also produced impairment of retention of passive avoidance. In one of these reports [4] a directly related dose-dependent deficit was found; in the second one, as in the present study, the amnesic effect was only obtained when atropine was injected into the anterior caudate but not when applied to the posterior aspect of this structure [11].

Behavioral effects of anticholinergic drugs applied to the ACN are not restricted to passive avoidance. Significant impairments in performance are also seen in active avoidance [7] and in a variety of positively reinforced tasks such a maze learning [12], lever pressing under a continuous reinforcement schedule [12,13,], spatial alternation [8] and autoshaping [1]. In contrast, application of anticholinergic agents in other regions of the brain are ineffective in producing these deficits (e.g. cerebal cortex [1,13], amygdala [10], hippocampus [5], cerebral ventricles [12], posterior [11] and ventral anterior aspects [7] of the caudate nucleus).

It is worth noting that the group of animals that was injected with atropine into the parietal cortex 2 min after training showed a small but reliable impairment. The retention score of this group differed from the scores of the unimplanted and the CN-30′ groups. However, the deficit was a minor one with respect to the caudate group that was similarly treated at 2 min. The same was also found to be the case in one of the experiments mentioned above [4]. It thus seems that for this particular task, cholinergic activity of the parietal cortex may play a role (albeit a minimal one) in memory.

One could speculate about the possiblity that our results were not due to interference with memory processes but rather to interference with perceptual, motivational, motoric or other related variables. We believe that this is not the case because: (a) any potential interference with non-associative processes can be ruled out in explaining our results since the treatments were given after the learning experience, and (b) retention testing was conducted 24 hr after training, when the direct effects of the anticholinergic drug had worn off. In other words, all animals were trained and tested in a non-drugged state.

In summary, the results of this experiment further support the view that cholinergic activity of the anterior caudate nucleus plays a critical role in memory processes.

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