Pharmacology Biochemistry & Behavior, Vol 24, pp 905–909, 1986 © Ankho International Inc Printed in the U S A 0091-3057/86 $3 00 + 00

Retrograde Amnesia Induced by Post-Trial Injection of Atropine Into the Caudate-Putamen. Protective Effect of the Negative Reinforcer

MAGDA GIORDANO* AND ROBERTO A. PRADO-ALCALÁ[1†]

**Laboratorio de Psicofisiología, Escuela de Psicología*
Universidad Anáhuac, México 10, D.F., México
and †Departamento de Fisiología, Facultad de Medicina
Universidad Nacional Autónoma de México
Apartado Postal 70250, México, D.F., México 04510

Received 27 March 1985

GIORDANO, M AND R A PRADO-ALCALÁ *Retrograde amnesia induced by post-trial injection of atropine into the caudate-putamen Protective effect of the negative reinforcer* PHARMACOL BIOCHEM BEHAV 24(4) 905–909, 1986 —A series of experiments was performed to test the reliability of previous reports which indicated that cholinergic blockade of the caudate-putamen produces memory deficits of passive avoidance, and to determine whether overtraining of this task protects against such deficits. In the first experiment the effects of different doses of atropine injected into the caudate-putamen of rats shortly after training were assessed, and a dose-dependent retention deficit was found In two additional experiments it was observed that by increasing the magnitude of the negative reinforcer used in training, a protection against such retention deficit was produced These results support the hypotheses that (a) cholinergic activity of the caudate-putamen is critically involved in memory processes that mediate passive avoidance behavior, and (b) after overtraining the control of this behavior is transferred from the striatal cholinergic system to other neurochemical systems within, or outside, the striatum

Caudate nucleus Caudate-putamen Striatum Neostriatum Memory Learning Retention
Passive avoidance Negative reinforcer Acetylcholine Atropine Overtraining

THE demonstration that lesions [4, 8, 11, 14, 24, 25, 28] and electrical stimulation [29, 30, 31] of the caudate-putamen (CPU) significantly impair the retention of passive avoidance conditioning has led to the investigation of the neurochemical events within the CPU, and related structures, that mediate this behavior. The role of striatal dopamine in passive avoidance is unclear since the neurochemical destruction of the ascending nigrostriatal dopaminergic pathway does not modify it [3] while application of this amine into the CPU produces retention deficits [7] Furthermore, electrical stimulation of the components of the nigro-neostriatal dopaminergic projection (substantia nigra and medial forebrain bundle) can facilitate and impair the retention of the aversively-motivated task [6, 10, 13, 26, 27]

The exploration of the involvement of the cholinergic system has yielded a clearer picture: interference with cholinergic activity of the CPU, induced after training or before testing, produces significant impairments in retention [5, 19, 23] which are both dose- and time-dependent [20,22]. On the other hand, application of the acetylcholine precursor choline into the CPU improves the performance of passive avoidance [2], and training of passive avoidance induces a significant increase in acetylcholine synthesis in this structure [1] These results strongly suggest that cholinergic activity of the CPU is critically involved in the acquisition and the maintenance of this aversively-motivated behavior

Related experiments have shown that application of anticholinergic drugs into the caudate of cats and rats interferes with the performance of newly learned instrumental behaviors and that after a period of overtraining there are no deficits in the performance of the same tasks [16,21]. It has also been found that while a generalized interference with the neural activity of the caudate, induced by direct injections of high concentrations of potassium chloride, induces a marked amnestic state, prolonged training also protects against this effect [18,21]. These data lend support to the hypotheses that

[1]Requests for reprints should be addressed to R A Prado-Alcalá, Dept of Physiology, Faculty of Medicine, National University of México, P O Box 70250, México, D F , México 04510 Supported by Consejo Nacional de Ciencia y Tecnología

(a) the striatal cholinergic system is critically involved in the acquisition and early maintenance stages of learning, but not in the performance seen after overtraining, and (b) after overtraining there is a transfer in the control of learned behaviors from the striatal cholinergic system to other neurochemical systems outside the caudate [16]

In all cases mentioned above, the overtraining-induced protection effect was investigated in experiments utilizing positively-reinforced tasks Thus, it was important to determine whether this effect is peculiar to that type of tasks or whether it reflects a more general mode of functioning of the nervous system The data presented in the present series of experiments confirm previous results [5, 19, 20, 22] and new findings demonstrate that the retention of an aversively motivated task can also be protected from the disruptive effects of cholinergic blockade of the CPU by manipulating variables involved in training

METHOD

Animals

Experimentally naive male Wistar rats, weighing between 250 and 350 g were used They were individually housed and had free access to solid food and tap water in their home cages. Under Nembutal anesthesia (50 mg/kg) double-walled cannullae were bilaterally implanted in the CPU, as described elsewhere [22] These animals were allowed 6–8 days to recover from the surgical procedures before training was initiated A group of unimplanted animals was also studied

Apparatus

Training and testing were carried out in a box (Lafayette Inst. Co , model 85000) with two compartments of the same size, separated by a guillotine door The grid floor of one of the compartments could be electrified by a square-pulse stimulator connected in series with stimulus isolation and constant current units (Grass Med Inst , models S-44, SIU-5A and CCU-1A, respectively). The opposite compartment had a wire mesh floor and its walls were painted blue Each of the compartments was equally illuminated by low intensity light bulbs

Procedure

Training and testing During training each animal was put inside the painted compartment of the conditioning box; ten sec later the door between compartments was opened and the latency to enter the gridded compartment with all four paws was measured. Once in the second compartment the door was closed and a footshock (60 msec pulses, 100 pulses per sec) was applied through the grid for five sec and the door was reopened, thus allowing the animal to escape to the first compartment and to remain there for 30 sec before being put back in its home cage Footshock intensities will be specified in each of the experiments

Twenty-four hr later a test session was programmed exactly as the training session, except that the footshock was not delivered If a rat did not cross within 600 sec to the compartment where the footshock had been given the session was ended and a score of 600 was assigned

Treatments

One injection of atropine sulphate (Sigma), dissolved in 3 μl of isotonic saline solution, was made through the implanted cannulae two min after training, to rats of independent groups The injection procedure was carried out in a room different from the room where training and testing took place All infusions were bilateral, in a volume of 3 μl through each cannula, delivered at a rate of 1 μl/20 sec, after injecting the solution the injectors were left inside the cannulae for an additional min During the procedure the rats were not restrained by the experimenter and could move freely in their home cages, thus avoiding stress reactions that could confound the results of the experiment

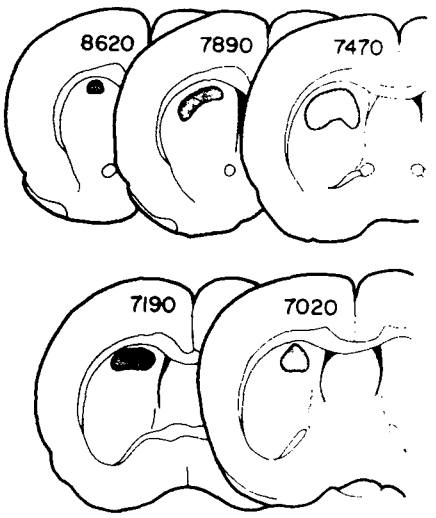

FIG 1 Diagramatic representation of histology sections, redrawn from Konig and Klippel [9] The stippled sections represent the range of cannula tip locations taken from the rats of the three experiments Only cannula placements of the right hemisphere are represented

Histology

At the completion of the experiment all implanted rats were deeply anesthesized and then perfused, intracardially, with isotonic saline followed by 10% Formalin, their brains were excised and kept in Formalin for at least one week before coronal sections (50 μm thick) were made and stained with the Nissl method to determine the location of cannulae tips

Statistics

The Barlett test was computed on latency scores for all groups during the training and the retention sessions The results showed that there was homogeneity of variances among the groups, this outcome allowed the use of parametric statistics The Fisher test (one-way ANOVA) was used to compare the latency scores and escape latencies among the groups and the Student's t test was used, when appropriate, to compare performances between each group and each of the other groups

EXPERIMENT 1

It has been demonstrated that injections of acetylcholine receptor-blockers into the CPU produce retrograde amnesia of passive avoidance [5, 19, 22] To our knowledge, there is only one report where the effects of different doses of an

anticholinergic drug (atropine) on the retention of passive avoidance were studied; a dose-related deficit in retention was found [20]. Therefore, it was important to determine the reliability of this result by testing the effects of other doses of atropine injected into the CPU shortly after training This experiment was also important in that it would allow to determine the appropriate dose levels that were to be used in the two additional experiments of this series.

Treatments

One of four doses of atropine sulphate (Sigma) was injected, two min after training, to independent groups through the implanted cannulae, the doses were 30, 45, 60, and 90 μg/3 μl (the doses refer to the salt). A group of unimplanted rats was also studied. Each group had 8 animals, except for the 90 μg group which had 9 animals Footshock intensity used for training was 0.25 mA

RESULTS AND DISCUSSION

The histological analysis revealed that the cannulae tips had been lodged in the antero-dorsal aspect of the CPU, rostral to the last trace of the anterior commissure (Fig. 1). The same result was obtained in Experiments 2 and 3

The analysis of variance indicated that there were no significant differences among the groups with regard to both latencies to step into the gridded compartment and escape latencies during the training session This outcome allowed us to use as a retention score for each animal a differential score, subtracting the latency score of the test session from the latency score of the training session A high score, especially one approaching 600, reflects good retention of the task whereas low or negative scores reflect an impairment in retention. As seen in Fig 2, highly significant differences appeared when these retention scores were compared, $F(4,36)=7\ 02$, $p<0.0001$. When each group was compared against each of the other groups (t tests) it was found that the animals treated with 30 or 45 μg of atropine and the unimplanted animals did not differ from each other, the same was true when retention scores were compared between the groups injected with 60 or 90 μg of atropine On the other hand, the 60 μg group, as well as the 90 μg group, differed significantly from the unimplanted, the 30 μg, and the 45 μg groups (p's$<0\ 05$).

The present results confirm those recently published [20] where a dose-dependent retention deficit of passive avoidance was produced by atropine. The present experiment was extended to test additional doses of the anticholinergic drug The fact that the lower doses do not interfere with retention while the higher doses do, indicate that there must be a critical number of cholinergic synapses that must be blocked in order to impair the consolidation process Other studies where only one dose of a cholinergic blocker was tested have consistently shown that there is a marked impairment in the acquisition and early maintenance stages of instrumental behaviors such as the passive avoidance task described here [5, 19, 22], active avoidance [12,15], lever pressing [16,21], etc In contrast, the injection of the acetylcholine precursor choline into the caudate produces an improvement in the performance of both negatively and positively motivated learned tasks [2, 15, 17]. Taken together, these data strongly suggest that instrumental conditioning is mediated by the caudate cholinergic system It is important to note that the doses of anticholinergic drugs that significantly impair retention of passive avoidance when applied to the anterior CPU produce a smaller retention deficit, or no deficit at all, when applied to the cerebral cortex [20,22], hippocampus [5] or posterior CPU [19]. The latter set of results indicate that retention of passive avoidance is dependent upon cholinergic activity of the anterior aspect of the CPU

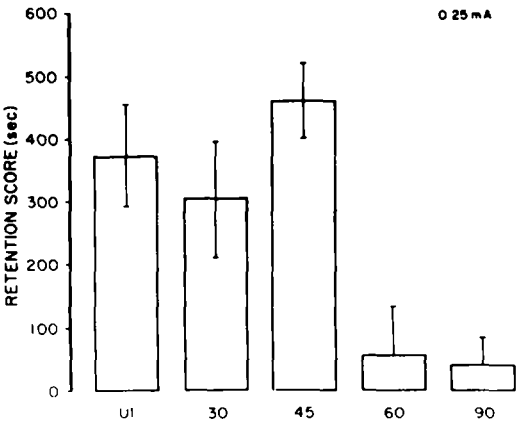

FIG 2 Mean retention scores (±SEM) shown by the unimplanted rats (UI) and by the rats with cannulae implanted in the caudate-putamen and treated, 2 min post-training, with 30, 45, 60 or 90 μg of atropine

EXPERIMENT 2

The aim of this experiment was to determine if overtraining of passive avoidance could protect the animals against the disruptive effects on memory produced by cholinergic blockade of the caudate-putamen Overtraining, as studied in the experiments referred to in the introduction, involved several factors multiple training sessions, a high number of positive reinforcers, and a prolonged exposure to the experimental situation [16, 18, 21] Because of the nature of the one-trial passive avoidance task used in our experiments, it is difficult to manipulate these variables There is only one trial, application of only one reinforcer (footshock), and the duration of the trial is brief To overcome this problem we decided to vary the magnitude of only one of these parameters—the reinforcer—by testing different intensities of footshock, keeping all other factors constant. This manipulation would be equivalent to having different amounts of positive reinforcers, as in the previous experiments.

Treatments

Three different footshock intensities were used: 0 25, 0.50, and 1.00 mA, two groups of rats were trained with each of these intensities (one group with cannulae in the CPU and one unimplanted group); each group was composed of 8 rats. All implanted rats were injected, 2 min after training, with the smallest dose of atropine that produced a significant impairment of retention in Experiment 1, i.e , 60 μg/3 μl.

RESULTS AND DISCUSSION

The analysis of variance indicated, as in Experiment 1, that there were no significant differences in latency scores nor in escape scores among the groups during the training session Hence, the retention score for each animal was computed as described above. Highly significant differences

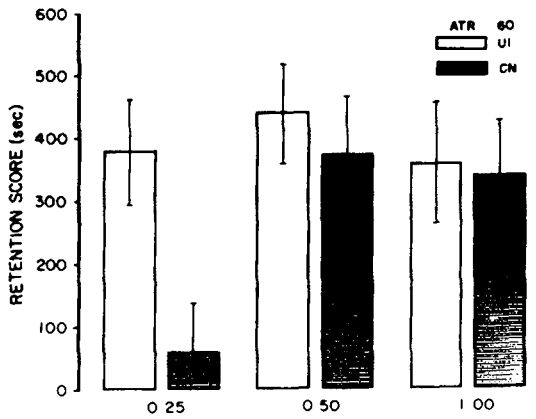

FIG 3 Mean retention scores (±SEM) shown by the groups that received a footshock of 0 25, 0 50 or 1 00 mA during training Open bars represent the data yielded by intact, unimplanted animals (UI), dark bars represent the data yielded by animals injected with 60 μg of atropine into the caudate-putamen (CN) 2 min after training

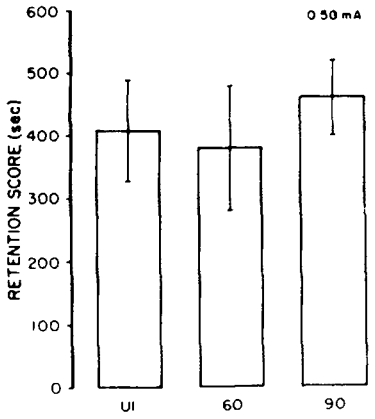

FIG 4 Mean retention scores (±SEM) of the groups of rats that were trained with a footshock of 0 50 mA and injected into the caudate-putamen with 60 or 90 μg of atropine UI refers to a group of unimplanted rats

among the groups appeared when these retention scores were compared, $F(5,42)=2\ 59$, $p<0.05$ Pair-wise comparisons revealed that only the striatal group that was injected with the atropine and trained with 0.25 mA had a retention deficit, as compared with each of the unimplanted groups This group also differed significantly from the striatal groups trained with 0.50 and 1 0 mA (p's$<0\ 05$ for all comparisons). None of the rest of the groups differed from each other; Fig 3 depicts these results

This is the first report where systematic changes in the magnitude of a negative reinforcer induces a protection against memory impairments that are produced by central administration of an anticholinergic agent The fact that the unimplanted groups of rats had similar retention scores, regardless of the intensity of the footshock with which they were trained, reveals that the lowest intensity (0 25 mA) was sufficient to produce learning as efficiently as the higher intensities The fact that only the striatal animals that were trained with 0.25 mA and treated with the atropine showed a memory deficit, suggests that only in these animals the striatal cholinergic activity was critically involved in the processes that mediate the retention of the passive avoidance task. In the case of the striatal animals that were trained with 0.50 and 1 0 mA it can be postulated that cholinergic activity of the CPU is less involved in these processes, or not involved at all

The memory deficit that was seen in the 0 25 mA group that was treated with atropine cannot be attributed to interference with sensory, motivational or motor activities since (a) the drug was given after training had taken place and testing was carried out 24 hr later, i.e , all treated animals were trained and tested in a drug-free state (this argument also holds for the results of Experiment 1), and (b) if the performance deficit had been due to an interference with non-mnemonic variables, then the same retention deficit should have been seen in the groups similarly treated but trained with the higher intensities of footshock (0 5 and 1 0 mA), such was not the case.

EXPERIMENT 3

In order to increase our confidence that cholinergic activity of the CPU becomes less involved, or not involved at all, in the retention of passive avoidance that had been trained with a high value of a negative reinforcer, it was necessary to explore the possibility that protection against memory deficits would still be found after a greater population of cholinergic synapses had been blocked

Treatments

All animals were trained using a 0 50 mA footshock and the implanted rats were injected with 60 (n=8) or 90 (n=6) μg of atropine Their performance was compared to that of an unimplanted group (n=8)

RESULTS AND DISCUSSION

The analysis of variance on latency and escape scores of the training session showed that the groups did not differ from each other Comparison of the retention scores among the groups also revealed that there were no significant differences (Fig 4)

The results of Experiments 2 and 3 showed that by increasing the intensity of the negative reinforcer the animals are protected against the retention deficit that is seen when atropine is given to animals that are trained with a low value of the reinforcer It was further shown, in Experiments 3, that the protective effect is manifest even when a high dose of atropine is injected into the striatum

To summarize, overtraining-induced protection against mnemonic deficits (of tasks where positive reinforcers were used) had been reported earlier, these deficits had been produced by cholinergic blockade and by generalized disruption of neural activity of the caudate [16, 18, 21] The present experiments demonstrate that the protective effect can be generalized to tasks mediated by negative reinforcers All these results support the hypotheses mentioned earlier, namely, that after overtraining (a) cholinergic activity of the caudate nucleus is not involved in the retention of instrumentally conditioned behaviors, and (b) there is a transfer of the control of instrumental behaviors from the striatal cholinergic system to other neurochemical systems within or outside the caudate nucleus

REFERENCES

1 Barker, L A , S D Glick, J P Green and J Khandelwal Acetylcholine metabolism in the rat hippocampus and striatum following one-trial passive training *Neuropharmacology* **21:** 183–185, 1982

2 Fernández, S M , M H. Solodkin and R A Prado-Alcalá Blockade and activation of caudate cholinergic activity *Soc Neurosci Abstr* **3:** 232, 1977

3 Fibiger, H C and A G Phillips Retrograde amnesia after electrical stimulation of the substantia nigra. Mediation by the dopaminergic nigro-neostriatal bundle *Brain Res* **116:** 23–33, 1976

4 Glick, S D and S Greenstein Comparative learning and memory deficits following hippocampal and caudate lesions in mice *J Comp Physiol Psychol* **82:** 188–194, 1973

5 Haycock, J W , S A Deadwyler, S I Sideroff and J L McGaugh Retrograde amnesia and cholinergic systems in the caudate-putamen complex and dorsal hippocampus of the rat *Exp Neurol* **41:** 201–213, 1973

6 Huston, J P and C C Mueller Enhanced passive avoidance learning and appetitive T-maze learning with post-trial rewarding hypothalamic stimulation *Brain Res Bull* **3:** 265–270, 1978

7 Kim, H -J and A Routtenberg Retention deficits following post-trial dopamine injection in rat neostriatum *Soc Neurosci Abstr* **2:** 631, 1976

8 Kirby, R J and D P Kimble Avoidance and escape behavior following striatal lesions in the rat *Exp Neurol* **20:** 215–227, 1968

9 Konig, F R J and R A Klippel *The Rat Brain A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem* Baltimore Williams and Wilkins, 1963

10 Le Piane, F and A G Phillips Differential effects of electrical stimulation of amygdala, caudate-putamen or substantia nigra pars compacta on taste aversion and passive avoidance in rats *Physiol Behav* **21:** 979–985, 1978

11 Mitcham, J C and P K Thomas Effects of substantia nigra and caudate nucleus lesions on avoidance learning in rats *J Comp Physiol Psychol* **81:** 101–107, 1972

12 Neill, D B and P S Grossman Behavioral effects of lesions or cholinergic blockade of dorsal and ventral caudate of rats *J Comp Physiol Psychol* **71:** 311–317, 1970

13 Phillips, A G and R Clouston Disruption of one-trial appetitive learning and passive avoidance following stimulation of the substantia nigra pars compacta *Behav Biol* **23:** 388–394, 1978

14 Polgar, S , P R Sanberg and R J Kirkby Is the striatum involved in passive avoidance behavior? A commentary *Physiol Psychol* **9:** 354–358, 1981

15 Prado-Alcalá, R A , G Cepeda, L Verduzco, A. Jimenez and E Vargas-Ortega Effects of cholinergic stimulation of the caudate nucleus on active avoidance *Neurosci Lett* **51:** 31–36, 1984

16 Prado-Alcalá, R A and G G Cobos-Zapiain Learning deficits induced by cholinergic blockade of the caudate nucleus as a function of experience *Brain Res* **138:** 190–196, 1977

17 Prado-Alcalá, R A. and G G Cobos-Zapiain Improvement of learned behavior through cholinergic stimulation of the caudate nucleus *Neurosci Lett* **14:** 253–258, 1979

18 Prado-Alcalá, R A and G G. Cobos-Zapiain Interference with caudate nucleus activity by potassium chloride. Evidence for a "moving" engram. *Brain Res* **172:** 577–583, 1979

19 Prado-Alcalá, R A , S E. Cruz-Morales and F A López-Miro Differential effects of cholinergic blockade of anterior and posterior caudate nucleus on avoidance behaviors *Neurosci Lett* **18:** 339–345, 1980

20 Prado-Alcalá, R A., M Fernández-Samblancat and M Solodkin-Herrera. Injections of atropine into the caudate nucleus impair the acquisition and the maintenance of passive avoidance *Pharmacol Biochem Behav* **22:** 243–247, 1985

21 Prado-Alcalá, R A , P Kaufmann and R Moscona Scopolamine and KCl injections into the caudate-putamen Overtraining-induced protection against deficits of learning *Pharmacol Biochem Behav* **12:** 249–253, 1980

22 Prado-Alcalá, R A , L Signoret and M Figueroa Time-dependent retention deficits induced by post-training injections of atropine into the caudate nucleus *Pharmacol Biochem Behav* **15:** 633–636, 1981

23 Sandberg, K , P R Sanberg, I Hanin, A Fisher and J T. Coyle Cholinergic lesion of striatum impairs acquisition and retention of a passive avoidance response *Behav Neurosci* **98:** 162–165, 1984

24 Sanberg, P R , J Lehmann and H C Fibiger Impaired learning and memory after kainic acid lesions of the striatum. A behavioral model of Huntington's disease *Brain Res* **149:** 546–551, 1978

25 Sanberg, P R , M Pisa and H C Fibiger Avoidance, operant and locomotor behavior in rats with neostriatal injections of kainic acid. *Pharmacol Biochem Behav* **10:** 137–144, 1979

26 Stabuli, U and J P Huston Effects of post-trial reinforcing vs subreinforcing stimulation of the substantia nigra on passive avoidance learning. *Brain Res Bull* **3:** 519–524, 1978

27 White, N and R Major Facilitation of retention by self-stimulation and by experimenter-administered stimulation *Can J Psychol* **32:** 116–123, 1978

28 Winocur, G Functional dissociation within the caudate nucleus of rats *J Comp Physiol Psychol* **86:** 432–439, 1974

29 Wyers, E. J and S A Deadwyler Duration and nature of retrograde amnesia produced by stimulation of caudate nucleus *Physiol Behav* **6:** 97–103, 1971

30 Wyers, E J , S A Deadwyler, N Hirasuna and D Montgomery Passive avoidance retention and caudate stimulation *Physiol Behav* **11:** 809–819, 1973

31 Wyers, E J , H V S Peeke, J S Elliston and M J Herz Retroactive impairment of passive avoidance learning by stimulation of the caudate nucleus *Exp Neurol* **22:** 350–366, 1968