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Is Cholinergic Activity of the Striatum Involved in the Acquisition of Positively-Motivated Behaviors?

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BERMÚDEZ-RATTONI, F , M MUJICA-GONZALEZ AND R A PRADO-ALCALÁ *Is cholinergic activity of the striatum involved in the acquisition of positively-motivated behaviors?* PHARMACOL BIOCHEM BEHAV **24**(3) 715–719, 1986 —Cholinergic activity of the caudate-putamen (CPU) is crucial for the acquisition of aversively-reinforced behaviors (active and passive avoidance) To determine whether this activity is also involved in the acquisition of a positively-rewarded behavior, in the present experimental series the effects of scopolamine applications to the antero-dorsal or postero-dorsal aspects of the striatum on auto-shaping were assessed The auto-shaping procedure that was used allowed rats to learn to bar press at their own rate It was found that scopolamine injection into either region of the CPU produced a marked retardation in the acquisition of the conditioned behavior These results indicate that cholinergic activity of the striatum is critically involved in the early phases of positively-reinforced learning

Cholinergic activity Auto-shaping Striatum Positively-reinforced learning

DIFFERENT techniques used to produce interference with normal neural activity, such as electrolytic lesions [4, 6, 20], electrical stimulation [21, 22, 23], injections of potassium chloride [14] and of neurotoxic chemicals [17,18], have been used to study the involvement of the caudate nucleus (CN) in learning processes In almost every case it has been found that such treatments produce marked impairments in conditioned responses

Other studies indicate that cholinergic activity of the caudate participates in these higher processes, cholinergic stimulation of this structure produces significant improvements in the retention of previously learned behaviors maintained by both positive [11] and negative [10] reinforcers Cholinergic blockade of the CN, on the other hand, leads to marked impairments of the same types of behavior [9,13] Related experiments strongly suggest that the striatal-cholinergic system is also involved in the acquisition of aversively-motivated responses, namely, passive [2, 5, 12, 15] and active [8] avoidance A novel approach to the study of the involvement of cholinergic activity of the striatum in memory processes has been recently described by Sandberg *et al* [19] A profound impairment in the acquisition and retention of passive avoidance was produced by the intrastriatal application of AF64A, a drug that produces a selective, long-lasting damage to cholinergic neurons

To date there are no published studies that clearly show that the cholinergic activity of the CN may be involved in the acquisition of positively-motivated behaviors The lack of such studies may only reflect the difficulty to design experiments in which potential interference with non-mnemonic variables can be ruled out The laboratory prototype of a positively-rewarded behavior is bar pressing conditioning Typically, the behavior of the animal is shaped by the experimenter, by the method of successive approximations, to approach and press the bar until a more or less sustained rate of responding is achieved Thus, the acquisition of this behavior is dependent upon both the learning capabilities of the animal and on the ability of the experimenter to shape the animal's behavior The latter variable is a major confounding factor in studies on the acquisition of conditioned responses

In the present study it was predicted that injections of an acetylcholine receptor-blocker into the caudate-putamen (CPU) would significantly impair the aquisition of a bar pressing response reinforced with water In order to avoid contamination in the acquisition phase of training that would otherwise be introduced by conventional shaping procedures, the animals of this study were trained using a modified auto-shaping procedure, which was based on the original paradigm of Brown and Jenkins [3], by which each rat learned the conditioned response at its own pace, without

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the direct intervention of the experimenter as previously described [1]

GENERAL METHOD

Animals

Experimentally naive male rats of the Wistar strain, weighing between 250 and 350 g were used They were individually lodged and had free access to solid food in their home cages Each animal was randomly assigned to one of four conditions (a) not implanted, (b) with cannulae bilaterally implanted in the antero-dorsal CPU, (c) with bilateral cannulae in the postero-dorsal CPU, or (d) with bilateral cannulae in the parietal cortex The cannulae for intracranial injections were implanted to the anesthetized (Nembutal, 45 mg/kg) animals as described elsewhere [15] The implanted rats were allowed seven days to recover from the surgical procedures before training was initiated There were 8 rats in each of the original groups that were to be studied (see below), but several animals were discarded because of apparatus failure, misplacing or dislodging of cannulae, or death

Apparatus

Two modified Skinner boxes (BRS/LVE model 143-24) were used, each was provided with two 8-watt light bulbs, one placed 2 cm above the bar that was located on the right hand side of the intelligence panel, 5 cm from the floor, and the other in the ceiling of a 4×4×4 cm niche with a dipper that could dispense water, located in the middle of the intelligence panel, 1 5 cm above the grid floor Environmental noise was masked by using an audio generator (BRS/LVE model AU-902) and by placing the conditioning box inside a sound-attenuating cubicle Control of light stimuli, delivery of water (reinforcer), and counting of pressing rates were accomplished with programmed electromechanical equipment located in an adjacent room

Procedure

All animals were deprived of water for about 23 hr before each session and had free access to tap water, in their home cages, during 30 min after each session During the first session each rat was given 50 trials of "dipper training," each trial was initiated by turning on the niche light for 4 sec, followed by the activation of the dipper mechanism, thus providing 0 05 ml of tap water There was a fixed inter-trial interval (ITI) of 30 sec during which all lights were turned off By the 25th trial all animals had learned to obtain water by poking their heads into the niche each time the light was turned on

Twenty-four hr later, and during four consecutive days, all animals were submitted to an auto-shaping procedure At the beginning of each trial the light above the bar was turned on for 30 sec, if the animals pressed the bar within those 30 sec the light was turned off and the cubicle light was simultaneously turned on for 4 sec and the dipper was activated, allowing the animals to obtain the reinforcer If the animals did not press the bar during the initial 30 sec period, the light above the bar was turned off and a 30 sec ITI ensued This sequence was repeated until 50 trials had been completed

Treatments

Each of the rats with cannulae implantation received only one injection through each cannula (see below) Two substances were used scopolamine bromide (Sigma), dissolved in isotonic saline (30 μg/3 μl) or isotonic saline alone (3 μl) It has been shown that while the dose of scopolamine that was chosen produces significant retention deficits in bar-pressing behavior it does not produce any noticeable interferences with motor activity or water intake [9] The solutions were delivered at a rate of 1 μl/20 sec through a 27 gauge needle connected to a microliter syringe (Hamilton) mounted on a slow-infusion pump (Sage, model 355) After injecting the solutions the 27 gauge needles were left inside the cannulae for an additional min During this procedure the animals were not restrained by the experimenter and could move freely in their home cages, thus avoiding stress reactions that could interact with the effects of the treatments

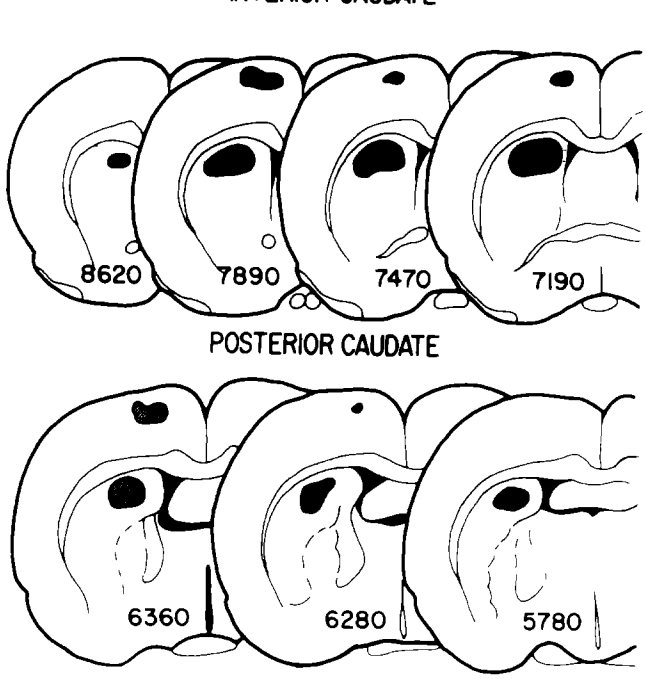

FIG 1 Diagramatic representation of histology sections, redrawn from Konig and Klippel [7] The stippled areas represent the range of cannula tip locations taken from the 32 rats of the dorsal-anterior striatal groups, 21 rats of the dorsal-posterior striatal groups, and the 22 animals of the cortical groups of Experiments 1 and 2 Only cannula placements of the right hemisphere are represented

Statistics

One-way ANOVA's (F test) among groups were computed on the number of reinforced bar press responses in each session When appropriate, comparisons of pressing rates between each group and each of the rest of the groups were made with the use of Student's *t*-test In every case the null hypotheses were rejected when values of less than 0 05 were obtained (one-tailed)

Histology

After completion of the experiments all implanted animals were deeply anesthetized with Nembutal and perfused, intracardially, with isotonic saline followed by 10% Formalin, their brains were excised and coronal sections (50 μm

thick) were made and stained (Nissl method) to determine the location of cannulae tips

EXPERIMENT 1

We were interested in determining whether bar pressing behavior could be acquired through auto-shaping in a condition where training was carried out under cholinergic blockade of the CPU To this end, scopolamine was injected into the antero-dorsal CPU (n=8), the postero-dorsal CPU (n=5), or the parietal cortex (n=6) of independent groups of rats Three additional groups were studied injected with isotonic saline into the anterior CPU (n=8) or into the posterior CPU (n=6), and a group of unimplanted animals (n=7) The treatments were given 6 min before the first auto-shaping session, i e , one day after dipper training

RESULTS

The histological analysis demonstrated that, as shown in Fig 1, all cannulae tips of the antero-dorsal CPU animals had been lodged in the antero-dorsal aspect of the caudate-putamen, rostral to the last trace of the anterior commissure, or in the postero-dorsal aspect of this structure, in those rats of the postero-dorsal groups The tips of the cortical cannulae were located in the parietal cortex, within the antero-posterior limits of the cannulae placements of the anterior CPU groups The same was true for the operated animals of Experiment 2

Figure 2 shows the number of bar presses across the auto-shaping sessions The analyses of variance showed that there were significant differences among the groups during each of the three first auto-shaping sessions, $F(5,34)=2\ 49$, $p<0\ 05$, $F(5,34)=8\ 60$, $p<0\ 01$, and $F(5,34)=5\ 63$, $p<0\ 01$, respectively Pairwise comparisons (t-test) indicated that there were no significant differences in response rates among the main control groups (antero-dorsal and postero-dorsal CPU groups treated with saline and the unimplanted group) in any of the three first sessions By the same token, no reliable differences between the two CPU groups that were treated with scopolamine were found However, each of this latter two groups had significantly lower scores when compared with the saline-treated and unimplanted groups during each of the first three auto-shaping sessions (p's$<0\ 05$)

An unexpected finding was that the cortical group did not differ from any of the other groups during the first session, differed reliably on the second session from the two CPU groups that had been treated with scopolamine, and by the third session it only differed from the group that was injected with scopolamine in the posterior CPU (p's$<0\ 05$) No data from the anterior CPU groups that had been treated with scopolamine and saline are shown in Fig 2, the animals of these groups were mistakenly sacrificed after the third auto-shaping session For this reason no statistical analyses were carried out on the fourth session, but it may be observed that the posterior CPU group that had been injected with scopolamine still had a lower pressing rate than each of the other groups

EXPERIMENT 2

Although Experiment 1 showed that there was a retardation in the acquisition of the instrumental task in the two striatal groups that had been injected scopolamine, interpretation of this finding is not without problems Since the treatment was given just before the first auto-shaping session, the animals were still under the effects of the scopolamine during this session Thus, it could be argued that the impairment in acquisition might have been due not to an interference with learning processes but, rather, to interference with motor, perceptual or motivational processes Furthermore, since these animals were dipper-trained in a normal state and tested for auto-shaping under the effects of scopolamine, the impairments that were seen could also have been due to a state-dependent effect It was important, therefore, to ascertain whether in the case of the acquisition of the bar press response cholinergic blockade of the CPU also produced its effect by disrupting associative processes, and not simply through interference with non-mnemonic processes The procedure employed in this experiment allowed discarding any such interference

One of the main factors upon which auto-shaping behavior is dependent is the association of conditioned-

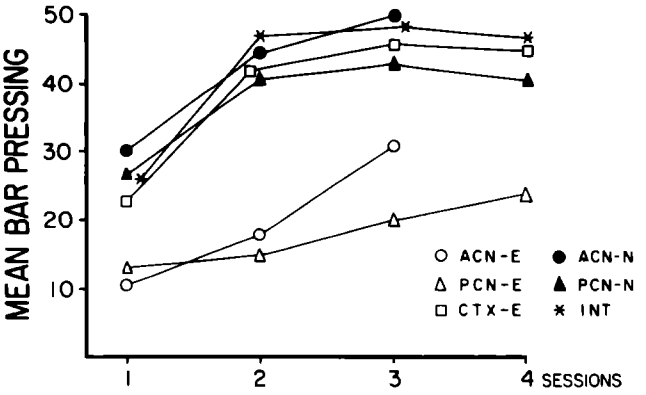

FIG 2 The ordinate represents the mean reinforced bar pressing rates displayed by the groups of rats that were injected with scopolamine into the anterior (ACN-E) or posterior (PCN-E) caudate-putamen nucleus or into the parietal cortex (CTX-E), by the isotonic saline-injected groups (antero-dorsal caudate, ACN-N, postero-dorsal caudate, PCN-N), and by the unimplanted, intact (INT) group Treatments were given 1 min after dipper training The ACN-E and the ACN-N groups were trained for only three sessions See text for details

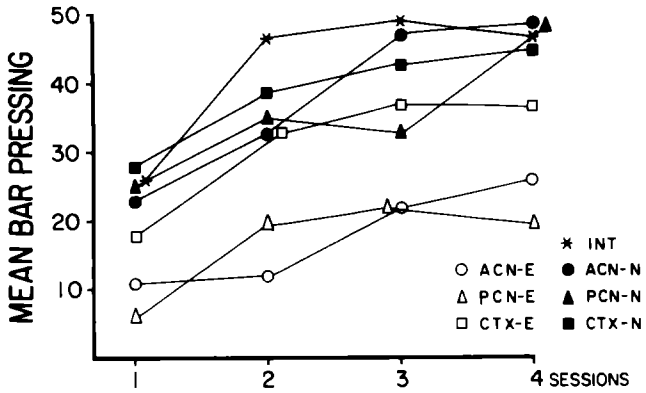

FIG 3 Mean reinforced bar pressing rates of the groups of rats injected with scopolamine or isotonic saline solution 6 min before the first auto-shaping session CTX-N, rats with cannulae implanted in the parietal cortex and injected with isotonic saline, the rest of the abbreviations represent the same as in FIG 2

unconditioned stimuli (light-water) presented during the dipper training session. Therefore, in order to assess the effect of scopolamine injection into the CPU on the acquisition of auto-shaped bar pressing, this drug was injected shortly after dipper training. Thus, all animals were in a drug-free state during initial training as well as during subsequent testing. The experimental procedure was the same as in Experiment 1, except that the only bilateral injection of scopolamine (30 μg/3 μl) or isotonic saline solution (3 μl) was delivered through the implanted cannulae 1 min after the dipper training session. Each experimentally naive rat was randomly assigned to one of the following groups: injected with scopolamine into the antero-dorsal CPU (n=8), the postero-dorsal CPU (n=5), or the parietal cortex (n=8), injected with isotonic saline into the antero-dorsal CPU (n=8), the postero-dorsal CPU (n=5), or the parietal cortex (n=8), a seventh group was composed of unimplanted animals (n=7).

RESULTS

Figure 3 depicts the learning curves derived from each of the groups that were studied. The analysis of variance indicated that there were significant differences in bar pressing rates among the groups during each of the four auto-shaping sessions, $F(6,39)=2.54$, $p<0.04$, $F(6,39)=3.34$, $p<0.009$, $F(6,39)=3.16$, $p<0.02$, and $F(6,39)=3.93$, $p<0.005$, respectively. Consistent with the findings in Experiment 1, the group of unimplanted animals did not differ significantly from the saline-treated rats (anterior CPU, posterior CPU and cortical groups), but differed on each of the four sessions from the anterior and posterior striatal groups injected with scopolamine (p's<0.05). The cortical group that was injected with scopolamine did not differ from the unimplanted group nor from the cortical group injected with saline solution on any of the sessions, it only differed, on session two, from the anterior CPU animals treated with the cholinergic blocker ($p<0.05$).

DISCUSSION

The results of Experiment 1 clearly show that administration of scopolamine to either the anterior or posterior striatum produces significant impairments in the acquisition of the bar press response induced through auto-shaping. These results may be explained as follows. Although the association between the cue light and the reinforcer had been established during the dipper training session (as evidenced by the consummatory response displayed by all animals in response to the cue light during this first session), the injections of scopolamine into the anterior and posterior CPU, applied before the first auto-shaping session, produced a marked interference with the retrieval of the learned association. This interference prevented the animals from responding appropriately to the cue light that was presented in the auto-shaping sessions. In other words, the animals that were tested under cholinergic blockade of the anterior or posterior CPU behaved as if they had not had dipper training. Their sustained low rates of responding throughout the experiment and the progressively increasing rates shown by the rest of the groups reflect the importance of the generalization from the first (niche) light cue to the second (bar) cue that must take place during the first auto-shaping session.

A similar interfering effect on retrieval, produced by cholinergic blockade of the caudate, has been described. When acetylcholine receptor-blockers are injected into the anterior CN of cats or CPU of rats that had learned to bar press, a marked amnesic state is produced [9,14]. However, when this learned response is overtrained no retention deficits are produced by the same treatments [9,14], thus indicating that in the cases where there was a decrement in responding it was not due to an interference with non-mnemonic variables. The lack of effects of the anticholinergics on memory in overtrained animals also suggests that state-dependency does not account for the amnesia that is seen in less trained animals. Consistent with this idea is a recent finding where passive avoidance training was given to drug-free rats which were later tested under cholinergic blockade of the CPU, these animals had as good retention scores as untreated animals [16].

The results of Experiment 2 agree well with those of the first experiment. In this case, a marked impairment in the acquisition of the bar pressing response was induced by the injection of scopolamine, shortly after dipper training, into the anterior or posterior CPU. The disruptive effects of scopolamine on auto-shaping behavior are interpreted as being due to an interference with the memory processes upon which the acquisition of the task are dependent. Any effects on motor, motivational, perceptual or other non-associative processes can be ruled out because the treatments were given after the initial training (dipper training) had taken place and long before the first auto-shaping session was carried out, when the direct effects of the anticholinergic drug had worn off, this experimental situation also rules out state-dependency. The striking similarity of these results with those of Experiment 1 indicate that the impaired acquisition of the bar pressing task seen in Experiment 1 was also due to an interference with memory processes.

Also consistent with the hypothesis that cholinergic activity of the CN is critically involved in the acquisition of learning are studies related to avoidance conditioning. As mentioned in the Introduction, cholinergic blockade of the anterior CPU (but not of the posterior CPU or the hippocampus), induced a few minutes after training of passive avoidance, produces a marked impairment in retention of the task, measured 24 hr later [5, 12, 13, 15]. A recent study indicates that this impairment is due to an interference with consolidation processes and not to interference with short-term memory [16]. Further evidence supporting the hypothesis under consideration has been provided by Barker *et al* [2], they demonstrated that there was an increase in acetylcholine synthesis in the CPU of rats shortly after training of this avoidance task.

An interesting lack of functional dissociation of cholinergic activity between the anterior and posterior CPU was found in both experiments of this series, i.e., retardation in the acquisition of auto-shaping was produced when scopolamine was injected to either region of the striatum. This finding is at variance with those where differential effects on various types of tasks have been described. The first report of a differential effect of scopolamine applications to the dorsal and ventral CPU on active avoidance was published by Neill and Grossman [8]. Subsequent studies showed that cholinergic blockade of the antero-dorsal striatum significantly impairs the retention of passive avoidance and the performance of positively reinforced bar pressing, as mentioned above [5, 9, 13, 15], cholinergic blockade of the posterior CPU, on the other hand, is ineffective in altering those types of tasks [9,12]. The only reported instance where cholinergic activity of the posterior CPU was found to play a more important role than that of the anterior

CPU in the performance of a learned behavior is in two-way active avoidance [10]

The auto-shaping procedure that was used proved to be a very sensitive method for assessing the effects of the injections of scopolamine into the parietal cortex Regardless of the time of injection (either after dipper training or before the first auto-shaping session), these treated animals performed, on the average, half-way between the control groups and the striatal groups that had been injected scopolamine In experiments where scopolamine was injected to the same cortical area tested in this study no significant changes in the performance of a previously learned bar press response were seen [9,14] Taken together these results indicate that cortical (parietal) cholinergic activity plays a functional role in the acquisition of bar pressing conditioning, albeit not as importantly as striatal cholinergic activity does, and that it is not significantly involved in the performance of this behavior once it has been established

The study of the role of cholinergic activity of the striatum and the cortex in learning has revealed dynamic, progressive changes in its relative involvement in such process It was shown in the present experimental series that cholinergic blockade of both anterior and posterior CPU and of the parietal cortex produced an impairment in the acquisition of bar pressing conditioning Once this response reaches asymptotic levels, however, cholinergic blockade of neither the posterior CPU nor the cortex produces memory impairments, while blockade of the antero-dorsal CPU does [9] With further training, cholinergic blockade of the antero-dorsal CPU [9,14] does not modify that same learned response All these results lend strong support to the hypothesis that there is a transfer in the control of learned responses from the caudate cholinergic system (which would operate during the acquisition and early maintenance stages) to other neurochemical systems within or outside this nucleus (operative during overtraining) [14]

To conclude, it was shown that application of scopolamine into either the antero-dorsal or the postero-dorsal aspects of the striatum greatly interferes with the acquisition of a positively-reinforced behavior and that a smaller degree of impairment is produced by the application of that drug to the parietal cortex

REFERENCES

1 Alemán, V , J L Camacho, F Bermúdez-Rattoni and R A Prado-Alcalá Differential (35 S) incorporation into protein of different brain areas of the rat during a learning task *Behav Neural Biol* **36** 137–154, 1982

2 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

3 Brown, P L and H M Jenkins Auto-shaping of the pigeon's key pecking *J Exp Anal Behav* **11**: 1–8, 1968

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

7 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

8 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

9 Prado-Alcalá, R A , F Bermúdez-Rattoni, D Velázquez-Martínez and M G Bacha Cholinergic blockade of the caudate nucleus and spatial alternation performance in rats overtraining-induced protection against behavioral deficits *Life Sci* **23**. 889–896, 1978

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

11 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

12 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

13 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

14 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

15 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

16 Prado-Alcalá, R A , L Signoret-Edward, M Figueroa, M Giordano and M A Barrientos Post-trial injection of atropine into the caudate nucleus interferes with long-term, but not with short-term retention of passive avoidance *Behav Neural Biol* **42**. 81–84, 1984

17 Sanberg, P R , J Lehman 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

18 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

19 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

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

21 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

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

23 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