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Disinhibitory Effects of Intrahippocampal or Intrahypothalamic Injections of Anticholinergic Compounds in the Rat[1]

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ROSS, J. F., L. J. MCDERMOTT AND S. P. GROSSMAN. *Disinhibitory effects of intrahippocampal injections of anticholinergic compounds in the rat.* PHARMAC. BIOCHEM. BEHAV. 3(4) 631–639, 1975. – The effects of intrahippocampal or intrahypothalamic injections of anticholinergic compounds on operant responding were observed in a multiple schedule paradigm consisting of reinforced, punished, and nonreinforced components and on a punished ingestive passive avoidance task. The pattern of results suggests that cholinergic components of the hippocampus and hypothalamus mediate responding suppressed by nonreinforcement but not by punishment. The data are discussed with reference to Carlton's proposed central cholinergic inhibitory mechanisms.

Anticholinergics Hippocampus VMH Punishment Reinforcement

IT is widely believed that the suppression of behavior which results from nonreinforcement or punishment may be the result of activation of cholinergic pathways in the brain [4,47]. The hypothesis is well supported by numerous reports of disinhibitory effects of systemically administered anticholinergics in a variety of paradigms, including habituation [4], extinction [17,19], signalled and nonsignalled reinforcement [16, 18, 25], fixed interval (FI) [20], differential reinforcement of low rate (DRL) [3], and Sidman avoidance [1].

What is not so clear is where these postulated pathways may be located. Shute and Lewis [45,46] have described two ascending cholinergic projections which innervate mainly the limbic system and associated basal forebrain regions. Krnjevic [27] has similarly reported that acetylcholine (Ach) containing neurons are found mainly in the phylogenetically old regions of the brain.

McCleary [32] has suggested that the septum may exert important inhibitory influences on punished and nonrewarded behavior. This hypothesis is supported by numerous studies which have shown apparently disinhibitory effects in a variety of paradigms, including passive avoidance [31], FI [8], DRL [9], and extinction [43]. Investigations of the hypothesis that these effects might be related to an interference with cholinergic components of the septum have suggested that the septal influence on punished and nonrewarded behavior may be more complex than the global effects of large septal lesions suggest. Thus, Hamilton *et al.* [15] observed that atropine injections into the septum of cats reproduced the disinhibitory effects of septal lesions in a passive avoidance situation and also increased punished responding in a forced extinction paradigm, but failed to affect position-habit reversal learning (a test which has consistently shown perseverative responding after septal lesions). Subsequent investigations have consistently failed to demonstrate disinhibitory effects of intraseptal atropine or scopolamine on punished behavior in passive avoidance situations [14,24], but responding to DRL and signalled Sidman avoidance paradigms has been found to be increased (Kelsey and Grossman, unpublished). These observations suggest that some, but not all, of the effects of anticholinergic compounds on punished or nonrewarded behavior may be related to pathways which can be influenced by direct application of cholinergic compounds to the septum.

Carlton [4] has suggested that the hippocampus, which is intimately related to the septum via the fimbrial fornix, may constitute another critical link in the central cholinergic circuit which appears to determine responses to nonreward or punishment. This hypothesis is well supported by numerous studies which demonstrate that lesions in the hippocampus mimic the effects of systemically administered anticholinergics on punished behavior [21,26], DRL acquisition [6], responding during periods of signalled nonreinforcement [49], or extinction [22,37]. Carlton's hypothesis is also supported by a number of

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experiments which have shown that direct application of anticholinergic or cholinergic compounds to the hippocampus [25, 40, 51] produce disinhibitory effects on operant responding in various experimental paradigms.

We [39,41] have recently found that transection of the fornix, which causes a loss of acetylcholine esterase (AchE) and presumably Ach in the hippocampus [36], produced overresponding in DRL and Sidman avoidance paradigms, but did not increase punished ingestive behavior in a passive avoidance situation. Van Hoesen *et al.* [50] have similarly reported that fornix lesions did not modify punished behavior. These observations suggest that the cholinergic components of the hippocampus may play an important role in the suppression of nonrewarded behavior, but may not influence reactions to punishment.

Stein [47] has suggested that cholinergic components of the ventromedial hypothalamus (VMH) may be part of a neural system which mediates the suppressive effects of punishment. Lesions in the ventromedial hypothalamus result in impaired reaction to punishment in passive avoidance situations [23, 30, 44] and there is some evidence that atropine injections into this region produce disinhibitory effects on punished operant responding [30,34]. There effects were not, however, seen in all punishment situations and there is some question concerning their specificity since various unpunished behaviors were also affected [13,34]. The effects of intrahypothalamic injections of atropine (or lesions) on the reaction to nonreward has not yet been described.

The present investigation was undertaken to provide further evidence on the influence of cholinergic components of the hippocampus and hypothalamus on punished and nonrewarded behavior.

GENERAL METHOD

Animals

Thirty-one male albino rats of the Sprague-Dawley strain (Holtzman Co., Madison, Wis.) weighing 300–400 gm. at the time of surgery were housed individually in a constantly illuminated colony.

Surgical Procedure

Prior to surgery, all animals were intraperitoneally (IP) injected with atropine sulfate (25 mg/kg) to avoid respiratory complications. Surgery was performed under Nembutal anesthesia (65 mg/kg IP). Double-walled stainless steel cannulas were sterotaxically implanted bilaterally into the dorsal or ventral hippocampi of 15 rats. For 8 of the animals the tips of the implants were aimed at AP = 3.0, H = 2.2, L = 2.8, using coordinates from the Pellegrino and Cushman [35] atlas of the rat brain. For 7 of the animals the tips of the cannulas were aimed at AP = 2.4, H = −0.5, and L = 4.9. A unilateral cannula was implanted just above the VMH nucleus in 16 rats. The tips of the cannulas were aimed at AP = 5.8, H = −2.5, and L = 0.8, using the coordinates from the de Groot [7] atlas of the rat brain. The design of the cannulas and details of the surgical procedure have been described elsewhere [12].

Drug Administration

Prior to a drug or control test, the inner cannula was removed from its usual position inside the animal's head, cleaned, and its tip tamped 5 times into a thin layer of drug in crystalline form which was spread on a plastic surface. It is estimated that this resulted in the accumulation of 1.0–5.0 μgm of the drug. The cannula was returned to its usual position in the animal's head 7–10 min before the beginning of the behavioral test to give the drug time to diffuse into the surrounding tissue. On control days, the inner cannula was returned empty. All cannulas were cleaned prior to the application of the drugs and immediately after each behavioral test.

Histology

After completion of the experiments, all rats were killed with a lethal dose of Nembutal and perfused intracardially with isotonic saline followed by 10 percent Formalin. After fixation, 50 μm frontal sections were cut through the region of the implants. Every third section was mounted on a glass slide and stained with cresyl violet.

Statistical Analysis

The effects of the drugs were evaluated by comparing the mean performance on two-predrug control days with performance on the drug day and with performance on postdrug control days by means of a Wilcoxon matched-pairs signed-ranks tests, except when the group N was less than six. In this case a *t* test for repeated measures was used. All tests were 2-tailed. To equate the effects observed in animals with different control baseline, the effects were evaluated in terms of percent change from control behavior.

EXPERIMENT 1

The first experiment was designed to investigate the behavioral effects of intrahippocampal and intrahypothalamic injections of anticholinergic compounds in an experimental paradigm which permits the concurrent observation of intermittently rewarded operant responding, as well as responding which is suppressed by punishment or nonreinforcement.

METHOD

Animals

The subjects were four rats with bilateral cannula implanted in the dorsal hippocampus, four rats with bilateral cannula implants in the ventral hippocampus, and sixteen rats with unilateral cannula implants whose tips terminated just above the ventromedial nucleus of the hypothalamus (VMN).

Apparatus

Two operant test chambers (36 × 25 × 33 cm) (Lehigh Valley Electronics, Model 1417) housed in sound insulating cubicles (LVE Model 1417C) were used. The chambers were equipped with levers (LVE Model 1352) which required a vertical force of at least 15 g to operate the microswitch. The levers were mounted 3.8 cm above the grid floor and 2.5 cm to the right of the left wall. A food magazine was installed 11 cm to the right of the levers and was connected to a pellet feeder (LVE Model 1548) mounted on the outside of the cubicle. The feeders delivered Noyes pellets (45 mg). Electric current could be passed through the grid floor, consisting of 0.5 cm diameter

stainless steel rods, spaced 1.5 cm apart. The electric current was generated by a high voltage AC source with a 47 K resistor in series with the grids. The chamber illumination was provided by a 0.3 A 28 V DC light bulb, mounted in the ceiling cubicle. A 2.8 W bulb, mounted on the panel 5 cm above the bar, served as stimulus light. All programming and recording equipment for the experimental events were controlled by electromechanical circuits and digital counters located in an adjacent room.

Procedure

All animals were starved to 85 percent of their preoperative body weight and trained to press a lever for food rewards available according to a continuous reinforcement schedule. For 8 rats with hippocampal cannulas and 8 rats with VMH cannulas the contingencies were gradually changed until animals were responding on a multiple schedule which consisted of 15 min of VI-30 sec reinforcement, 3 min of punishment, 4 min of nonreinforcement, 15 min of VI-30 sec reinforcement, 3 min of punishment, 2 min of nonreinforcement. The VI schedule was indicated by the illumination of the houselights. During the punishment period, an indicator light was illuminated over the lever and each response was followed by both a food pellet and 0.5 sec of grid shock. Each animal was given a 42 min session each day until its performance was stable. The criteria for stability were reached when (a) the number of responses in the VI, punishment, and nonreinforcement periods for 2 consecutive days did not differ by more than 10 percent from the mean of the 2 days (if less than 10 responses were made during the punishment and nonreinforcement periods, a difference of 2 or fewer responses on 2 consecutive days was considered stable) and (b) no more than thirty responses were emitted during the nonreinforcement period. When these criteria had been met, the anticholinergic compounds, atropine methyl nitrate, an antimuscarinic agent, or mecamylamine hydrochloride, an antinicotinic agent (Sigma Co., St. Louis, Mo.) were administered to the VMH or hippocampus on the following day.

An attempt was made to adjust the shock intensity for each animal so that it was possible to administer drug treatments while they were responding both at relatively high (30 or more responses) and relatively low (fewer than 30 responses) rates during the punishment period. Most animals thus received each drug before 2 VI and 2 nonreinforcement sessions and statistical analyses were performed on the mean of the data for the two different drug days for these two periods.

Eight additional animals with hypothalamic cannulas were trained and tested in a similar manner except that the multiple schedule did not include a period of nonreinforcement. For these animals, the paradigm consisted of 15 min VI, 3 min punishment, 15 min VI, and 3 min punishment. For purposes of statistical evaluation, the VI and punishment data from these two groups of animals with VMH cannulas were combined.

RESULTS AND DISCUSSION

Anatomical

Figure 1 shows representative cannula placements from animals with (a) bilateral dorsal hippocampal cannulas, (b) bilateral ventral hippocampal cannulas, and (c) a unilateral

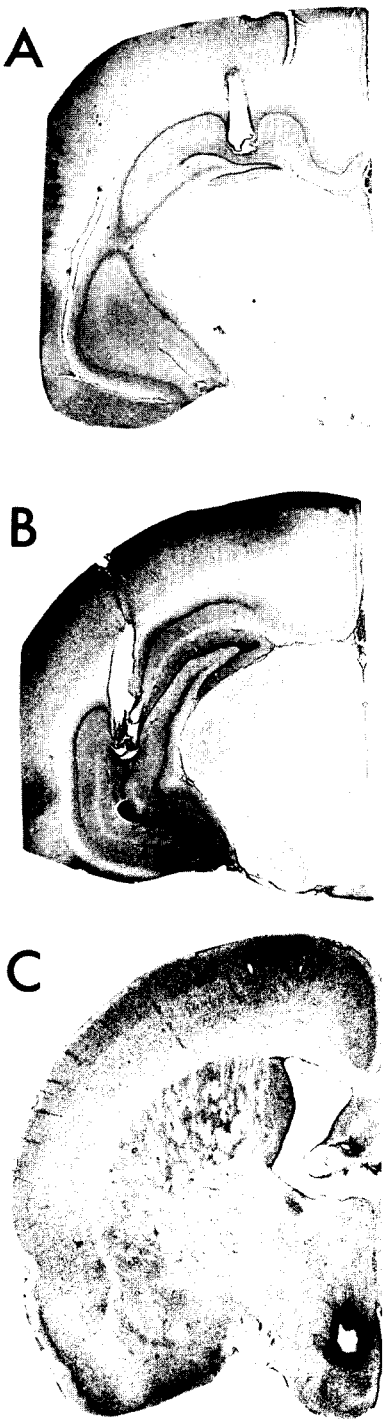

FIG. 1. Photomicrographs of coronal sections of rat brains, showing the position of cannulas used to apply atropine or mecamylamine to the (a) dorsal hippocampus, (b) ventral hippocampus, and (c) ventromedial nucleus of the hypothalamus.

cannula above the ventromedial nucleus of the hypothalamus (VMN). The majority of the dorsal hippocampal placements were localized in cell layers CA1 and CA2. Most of the ventral cannulas were localized in the stratum radiatum. Only data from those animals whose cannula placements fell directly above the VMN and did not damage the nucleus itself were used.

Behavioral Observations

Intrahippocampal administrations of atropine or mecamylamine reliably ($p \leq 0.02$) increased responding during the period of signalled nonreinforcement. Responding during the punishment or VI periods was not significantly ($p > 0.10$) affected by either compound. The disinhibitory effects of mecamylamine were no longer present on the first postdrug day. Atropine, on the other hand, reliabily increased nonreinforced responding ($p < 0.05$) on the first post-drug test but this residual effect disappeared on the on the second control test (see Fig. 2). Ventral and dorsal cannulas produced comparable effects of all drugs.

These results are in good agreement with previous observations from our laboratory that injections of atropine or mecamylamine into the hippocampus of rats increase nonreinforced responding [40]. Our present finding that these treatments do not disinhibit punished responding in the same situation suggests that cholinoceptive components of the hippocampus may selectively affect behavior suppressed as a result of nonreinforcement.

Intrahypothalamic injections of atropine, but not of mecamylamine, reliably ($p \leq 0.05$) increased responding during signalled nonreinforcement (see Fig. 3). Mecamylamine, but not atropine, also produced a small (9%) but statistically reliable ($p \leq 0.05$) increase in VI responding. Neither compound increased punished responding as we had expected on the basis of previous reports [30]. A closer look at our data (see Table 1) indicates that intrahypothalamic atropine injections, in fact, significantly decreased ($p < 0.05$, $t = 3.43$, $df = 5$) punished responding in all animals (N = 5) that had a relatively high baseline of responding during the punishment period. Thirteen of the 16 animals tested in these experiments showed a decrease or no change in punished responding after atropine injections in the hypothalamus. The 3 animals that increased responding on the drug test did so by no more than 4 responses. Two of these animals were in the group tested in the three-part multiple schedule and the other one was in the group tested in the two-part multiple schedule. For 8 of these animals, the test paradigm was essentially identical to the two-part schedule used by Margules and Stein [30] who found that approximately half of their animals displayed increased punished responding after intrahypothalamic atropine injections. The published account of the cannula placements does not provide obvious interpretations for the puzzling inconsistency of the effect in the experiments reported by Margules and Stein [30] and we have not been able to find substantial differences between our own placements and some that were labelled effective in their report. Nor does it appear likely that dosage differences account for our failure to obtain effects on punished responding since we (a) attempted to duplicate the dose used by Margules and Stein [30]; (b) observed significant effects on responding during VI and nonreinforcement periods of the schedule; and (c) have observed marked

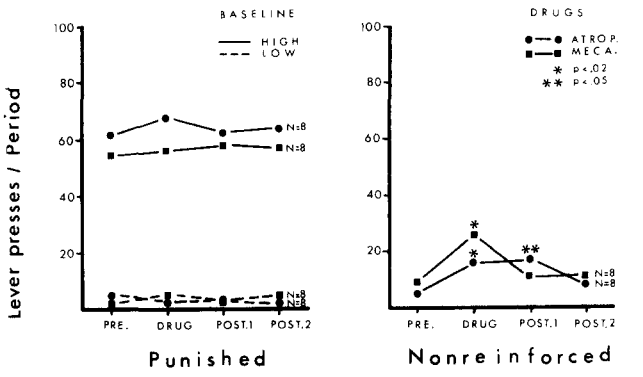

FIG. 2. Effects of intrahippocampal injections of atropine methyl nitrate or mecamylamine hydrochloride on lever-pressing in the punishment and nonreinforcement periods of a multiple schedule.

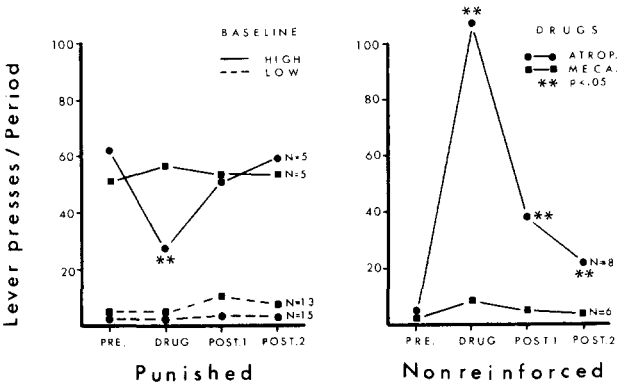

FIG. 3. Effects of intrahypothalamic injections of atropine methyl nitrate or mecamylamine hydrochloride on lever-pressing in the punishment and nonreinforcement periods of a multiple schedule.

effects of intrahypothalamic atropine on avoidance behavior in previous investigations using similar doses [13].

The effects of intrahypothalamic atropine on nonreinforced responding on both postdrug tests were markedly diminished but statistically reliable ($p < 0.05$). These persisting effects disappeared by the third or fourth day on the average. Such a long time course is unusual in our experience, but Margules and Margules [29] have reported behavioral effects of intrahypothalamic atropine injections which persisted for up to three weeks. The long term effects of their injections appeared not to be related to tissue damage since they were promptly reversed by physostigmine. The persistence of the behavioral responses to hypothalmic atropine injections in the present investigation is particularly interesting when contrasted with the time course of the effects of intrahypothalamic mecamylamine on VI responding (which were confined to the drug test itself) and with the reaction to intrahippocampal injections of mecamylamine or atropine on nonreinforced responding (which showed a tendency to persist in diminished fashion on the first postdrug day, but had disappeared entirely on the second) (See Table 2).

The pattern of effects seen after intrahypothalamic injections of anticholinergics (an increase of nonreinforced,

TABLE 1

EFFECTS OF INTRAHYPOTHALAMIC INJECTIONS OF ATROPINE ON RESPONDING DURING TWO 3-MIN PUNISHMENT SEGMENTS OF A MULTIPLE SCHEDULE

	Hypothalamic Injections			
Animal Number	Predrug	Atropine	Postdrug (1)	Postdrug (2)
Low Baseline				
489	1	0	1	0
491	3	3	5	2
493	1	1	1	1
497	2	0	1	1
494	2	1	1	1
495	1	3	2	1
496	0	1	2	2
492	1	1	3	3
15	3	7	2	3
16	5	4	11	5
17	4	3	4	7
19	3	3	2	2
20	3	0	3	3
21	2	0	3	14
22	1	0	0	2
High Baseline				
492	85	74	89	80
489	15	13	4	21
491	72	20	63	73
494	69	19	59	63
496	65	17	53	60

but not of punished responding) supports the earlier suggestion by Miczek and Grossman [34] that the cholinergic components of the ventromedial hypothalamus probably do not exert disinhibitory effects on behavior which are specific to punished responding. Our data suggest, in fact, that the cholinergic components of the hypothalamus, much like those of the hippocampus, may preferentially affect responding suppressed as the result of nonreinforcement. This does not, of course, compel the conclusion that the hypothalamic and hippocampal mechanisms are part of the same cholinergic inhibitory system. Closer inspection of the pattern of drug effects seen after intrahippocampal and intrahypothalamic injections shows a number of dissimilarities that indicate that we may be dealing with two functionally as well as anatomically distinct mechanisms which may exert somewhat different influences on reactions to nonreinforcement. Both (as well as cholinergic components of the septum) would, presumably, be affected by systemic injections of anticholinergics and it thus appears likely that the effects of intracranial injections at any of these sites may reproduce some, but not all, of the effects of systemic injections.

EXPERIMENT 2

In the first group of experiments, intrahypothalamic injections of atropine disinhibited nonreinforced responding but further suppressed punished responding in a multiple-schedule lever-pressing situation. Punished responding was also decreased or unaffected by intrahypo-

TABLE 2

EFFECTS OF INTRAHIPPOCAMPAL INJECTIONS OF ATROPINE ON RESPONDING DURING TWO 3-MIN PUNISHMENT SEGMENTS OF A MULTIPLE SCHEDULE

	Hippocampal Injections			
Animal Number	Predrug	Atropine	Postdrug (1)	Postdrug (2)
Low Baseline				
700	1	0	1	0
701	2	1	1	1
702	0	0	1	0
704	3	2	4	3
705	0	1	0	2
706	10	4	3	2
708	7	4	2	0
710	0	1	0	2
High Baseline				
700	36	68	77	71
701	92	96	82	84
702	63	68	59	63
704	37	40	35	39
705	64	57	60	69
706	68	71	73	74
708	64	63	60	65
710	60	67	55	55

thalamic atropine in a simpler paradigm which did not include periods of nonreinforcement. It is nonetheless clear that components of the ventromedial hypothalamus exert a strong influence on at least some types of punished behaviors since lesions in the area produce unequivocal passive avoidance deficits with respect to punished ingestive behavior [23,44]. Margules and Stein's [30] observations suggested that cholinergic components of the ventromedial region might be responsible for some of these effects. Miczek and Grossman [34] found that intrahypothalamic injections of atropine affected punished as well as unpunished responding in some experimental paradigms, but had little or no effect in others, suggesting that the influence of the cholinergic components of the VMH may be situation-dependent. It thus appeared important to conclude the present investigation of these mechanisms by observing the effects of intrahypothalamic or intrahippocampal atropine and mecamylamine injections on punished ingestive behavior in a passive avoidance paradigm which previously had shown significant disinhibitory effects of lesions in the VMH [44].

METHOD

Animals

Seven rats with hippocampal cannulas and 7 rats with cannulas above the VMN were used. The animals with the VMN cannulas had previously been tested in the preceding experiment in the multiple schedule paradigm consisting of the VI and punishment periods only.

Apparatus

Four test chambers (24 X 24 X 30 cm) were used. The front, back, and tops of the chambers were made of transparent Plexiglas and the sides were black Plexiglas. The floor of each chamber consisted of stainless mesh. A Wahman calibrated watering device was inserted into each chamber through a 2 cm dia. hole located in the front panel, 4 cm from the right side and 2.5 cm above the floor. A 480 V step-up transformer with a limiting series resistor was used to pass DC current through the rat whenever it completed the circuit between the mesh floor and the metal

TABLE 3

EFFECTS OF INTRAHYPOTHALAMIC OR INTRAHIPPOCAMPAL INJECTIONS OF ATROPINE ON PUNISHED INGESTIVE BEHAVIOR IN A PASSIVE AVOIDANCE PARADIGM

Animal Number	Predrug	Atropine	Postdrug (1)	Postdrug (2)
Hypothalamic Injections				
15	5	0	6	5
16	6	1	4	3
17	4	3	5	3
19	3	10	1	5
20	11	4	10	46
21	7	2	3	1
22	4	5	4	5
Hippocampal Injections				
451	3	1	12	6
435	14	21	7	8
453	3	6	8	9
337	3	2	1	1
452	22	1	8	5
450	3	3	1	1
444	8	6	4	4

drinking tube. Each such circuit closure was automatically recorded.

Procedure

All rats were reduced to 85 percent of ad lib body weight by restricted feeding. Each day the rats were placed into the test chamber and allowed free access to a 50:50 mixture of evaporated milk and an 8 percent glucose and water solution for 15 min. When the intake of an animal on 3 consecutive days did not vary by more than 10 percent from the mean of the 2 days, intracranial injections of atropine methyl nitrate or mecamylamine were administered as described in Experiment 1 above.

After the effects of the drug injections on fluid intake had been investigated, a second phase of training and testing was begun. Each day the rat was allowed free access to the solution for 2 min. During the remaining 13 min of the session, the metal drinking tube and the wire mesh floor were connected to the shock source so that the rat received a shock whenever it touched the tube with its tongue or snout while attempting to drink. When the number of punished responses on 2 consecutive days did not differ by more than 10 percent, the rat was again given intracranial injections of atropine or mecamylamine as previously described. Shock intensity was adjusted so that responding was suppressed to fewer than 10 responses per session in each animal.

RESULTS AND DISCUSSION

Anatomical

The locations of the cannula tips for the animals with hippocampal cannulas were similar to those seen in Experiment 1 (see Fig. 1). The locations of the cannula tips for the VMH animals have been previously described in the Results and Discussion of Experiment 1.

Behavioral Observations

Intrahippocampal injections of atropine or mecamylamine did not reliably increase punished licking (see Table 3). This is in good agreement with observations made in Experiment 1 and in a previous investigation [40]. Viewed together, our results indicate that cholinergic components of the hippocampus do not influence punished responding in a variety of situations. Atropine, but not mecamylamine, produced a small (36 ml predrug, 33 ml atropine) but statistically reliable ($p<0.05$) decrease in the unpunished intake of the palatable fluid. The drug effects on unpunished intake were not sufficiently consistent to indicate a meaningful relationship to the increase in the intake of

palatable fluids which has been seen after septal lesions as well as transections of the septo-hippocampal connections [39].

Intrahypothalamic injections of atropine or mecamylamine also did not produce significant disinhibitory effects on punished licking either on the day of the treatment or on subsequent control tests (see Table 3). Repeated testing with a wide range of doses of both compounds failed to demonstrate significant increases in punished responding after mecamylamine or atropine. Indeed, the majority of the animals (5 out of 7) consistently displayed a decrease in punished responding after intrahypothalamic atropine injections. The two animals who displayed a small increase in responding on the initial atropine test did not show this effect consistently on subsequent tests. The predominantly inhibitory effects of intrahypothalamic atropine on punished licking are in excellent agreement with the decrease in punished operant responding that was seen after intrahypothalamic atropine in the preceding experiment. Neither atropine nor mecamylamine injections into the VMH produced significant effects on unpunished intake of sweet milk. This observation replicates earlier reports [13,30] which indicate that the cholinoceptive components of the ventromedial hypothalamus do not influence ingestive behavior.

GENERAL DISCUSSION

The results of the present experiments are internally consistent in showing that intrahippocampal and intrahypothalamic injections of anticholinergic compounds increase nonrewarded responding, but have no disinhibitory effects on responding suppressed by punishment. The effects of the intrahippocampal drug treatments are in excellent agreement with our earlier observations which demonstrated that injections of anticholinergic compounds into the hippocampus produced inefficient overresponding in a modified DRL situation [40]. The present results are also congruent with the observation that fornix transections which reduce cholinergic activity in the hippocampus [36] also produced an increase of nonrewarded responding in our DRL paradigm [41], but did not increase punished responding in passive avoidance situations [39,50].

The results of the present experiments suggest that the disinhibitory effects on punished behavior which have been observed in animals with hippocampal lesions [21,26] may be due to an interference with non-cholinergic components of the hippocampus which may project to or through the entorhinal cortex where lesions also increase punished responding [10, 42, 50]. These connections with the entorhinal cortex have been shown to contain no cholinesterase [28].

Our results are in good agreement with Carlton's earlier suggestion [4] that the hippocampus may contain cholinergic mechanisms that are specifically related to the suppression of nonreinforced behavior. Carlton and Markiewicz [5] have more recently suggested that central cholinergic mechanisms may also mediate the suppressive effects of punishment in some situations. There is considerable evidence [1, 2, 11, 16, 33, 48] that the effects of anticholinergic compounds on responding which is suppressed by aversive stimuli are seen in some experimental paradigms (e.g., conditioned fear or step-down passive avoidance) and not in others (e.g., punishment of previously established operant responding or over-trained passive avoidance). The results of the present investigation suggest that the effects of anticholinergic compounds in these instances may not be mediated by hippocampal or hypothalamic mechanisms. It has previously been reported that intraseptal injections of anticholinergics increases punished ingestive and operant responding in some experimental paradigms and not in others [14, 15, 24, Kelsey and Grossman, unpublished]. In conjunction with the results of the present investigation, these observations suggest that at least some of the effects of systemically administered anticholinergics on punished responding may be related to their action on cholinergic components of the septum. Ross [38] has recently demonstrated that the effects of septal lesions on punished responding are reproduced by transections of the stria terminalis. It is thus possible that the anatomical substrate for the effects of intraseptally as well as systemically administered anticholinergics on some types of punished responding may be the bed nucleus of that pathway, or connections between the stria terminalis and the septum itself.

The results of the present experiments indicate that cholinergic components of the ventromedial hypothalamus also influence the suppressive effects of nonreinforcement, but not the response to punishment. Since the ventromedial nucleus itself contains little or no acetylcholinesterase [45], it is likely that the response to nonreinforcement is influenced by other structures within the ventromedial hypothalamus.

Our consistent failure to obtain disinhibitory effects of intrahypothalamic atropine injections on punished ingestive as well as operant behavior fails to replicate the observation reported earlier by Margules and Stein [30] that intrahypothalamic atropine injections increase punished responding reliably in some rats in a paradigm similar to that used in our first experiment. The reasons for this discrepancy are not apparent at this time. We [34] have previously found that intrahypothalamic atropine injections produce variable effects on punished as well as unpunished responding in the monkey and suggested these effects might not be related to a selective inactivation of cholinergic mechanisms that mediate the suppressive effects on punishment. The results of the present investigation are congruent with this interpretation.

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