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Lesions of Cholinergic Forebrain Nuclei: Changes in Avoidance Behavior and Scopolamine Actions

G. LO CONTE, L. BARTOLINI,* F. CASAMENTI
I. MARCONCINI-PEPEU AND G. PEPEU[1]

*Departments of Pharmacology and Neurology, *University of Florence*
Viale Morgagni 65, 50134 Florence, Italy

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LO CONTE, G., L. BARTOLINI, F. CASAMENTI, I. MARCONCINI-PEPEU AND G. PEPEU. *Lesions of cholinergic forebrain nuclei: Changes in avoidance behavior and scopolamine actions.* PHARMAC. BIOCHEM. BEHAV. **17**(5) 933–937, 1982.—The acquisition of active (shuttle-box) and passive avoidance conditioned responses and the effects of scopolamine on acetylcholine (ACh) output in freely moving rats and on conditioned responses were investigated 20 days after placing a unilateral lesion in the magnocellular forebrain nuclei (MFN). In the lesioned rats spontaneous ACh output from the cerebral cortex ipsilateral to the lesion was slightly decreased, while on the other hand the increase in ACh output elicited by scopolamine was strongly reduced. Sham operated rats always performed more active avoidance responses than MFN lesioned rats in the daily training shuttle-box sessions, and the facilitating effect of scopolamine (1 mg/kg IP) on the shuttle-box performance was suppressed. However the lesion did not disrupt the shuttle-box performance whenever training had taken place before the lesion. In the lesioned rats retested 30 min after the training trial, an impairment of the passive avoidance response was found. The effect of the lesion was potentiated by scopolamine. The results show therefore that MFN lesions impair the cortical cholinergic mechanisms, whose activity seems to play an important role in cognitive functions.

Magnocellular forebrain nuclei Scopolamine Acetylcholine release Active avoidance Passive avoidance

IT HAS recently been shown [15, 21, 22] that in the rat a large cholinergic pathway projecting to cortical areas originates from neurons located in the ventromedial portion of the pallidum (also known as nucleus basalis magnocellularis, or Maynert's nucleus basalis), the medial and lateral preoptic nuclei and the entopeduncular nucleus which lie within the so-called area innominata [22].

In the present paper these nuclei will be defined as magnocellular forebrain nuclei (MFN) [8,15]. Their unilateral destruction by kainic acid [13] or electrolytic lesions [17,22] is followed by a selective, marked decrease in cholinoacetyltransferase and acetylcholinesterase activity in the ipsilateral cortex, indicating the disappearance of cholinergic nerve endings. A decrease in spontaneous acetylcholine (ACh) output from the cerebral cortex ipsilateral to the lesion also occurs [16]. On the other hand cortical neurotransmitters, such as catecholamines, GABA and 5HT are not affected [13].

The impairment of the cortical cholinergic mechanisms is associated with a reduction in total electrocortical activity, a decrease in the shuttle-box performance and an increase in spontaneous motility [16].

In the present work the study of the behavioral effects of unilateral lesions of the MFN was extended using active and passive avoidance-conditioned responses, in order to detect whether both acquisition and retention were impaired. The comparison was also made between the effect of the lesions and that of scopolamine, so as to define the role in cognitive functions of the cholinergic pathway ascending to the cortex.

METHOD

Animals

Male Wistar rats, Nossan strain, 140–150 g body weight before the operation, were individually housed and given free access to food and water in the home cage.

Surgery

Under ketamine (Ketalar, Parke Davis) anaesthesia (100 mg/kg IP) unilateral lesions were made according to the following coordinates taken from the Koenig and Klippel atlas of the rat brain [14]: 0.2 anterior to bregma, 2.5 lateral, 7 mm below dura. A current of 1.0 mA was passed through a unipolar electrode for 30 sec. In 8 rats a smaller lesion aimed to destroy only the medial forebrain bundle was placed according to the coordinates: 1.1 posterior to bregma, 1.6 lateral and

[1]Requests for reprints should be addressed to Professor G. Pepeu, Department of Pharmacology, University of Florence, Viale Morgagni 65, 50134 Florence, Italy.

7 mm below dura. A current of 1.0 mA was passed for 10 sec. In the sham-operated rats the electrode was lowered into the cortex without passing any current. At the end of the experiments a histological examination was carried out on each animal in order to assess the placement and size of the lesion.

ACh Output

ACh output from the cerebral cortex was measured in unanaesthetized rats by a previously reported method [6]. Briefly stated, 20 days after placing the lesion, a disc of left or right parietal bone was removed under ketamine anaesthesia and a Perspex cylinder was screwed into the bone, so as to exert a slight compression on the dura mater, and secured with dentist cement. ACh output was measured three days after surgery. During this time the dura was washed with a terramycin solution (300 μg/ml). The Ringer solution placed in the collecting cylinder had the following composition: NaCl 9, KCl 0.42, $CaCl_2$ 0.24, $MgCl_2$ 0.05, glucose 2 g/l, physostigmine sulphate 100 μg/ml. Every 20 min the solution was removed from the cylinder and bioassayed for ACh on the dorsal muscle of the leech.

The identity with ACh of the active substance released in the cup under these experimental conditions has been repeatedly demonstrated [3,19]. The following criteria have been used: (a) when physostigmine was omitted from the solution in the cup, no active substance could be detected; (b) the cup samples and equiactive concentrations of ACh added to the Ringer solution were inactivated when they were treated with four drops of 0.1 NaOH, kept at room temperature for 30 min and neutralized with 0.1 HCl; (c) the samples were not active when d-tubocurarine chloride (Tubarine, Wellcome) 3×10^{-6} g/ml, was present in the solution bathing the leech muscle; (d) a linear dose-effect relationship parallel to that obtained with 2 to 10 ng of ACh could be constructed using different volumes of the samples.

Active Avoidance Conditioned Response

The acquisition of an active avoidance conditioned response was investigated in an automated, two-way shuttle-box [4] made by Basile (Comerio, Italy). The unit consisted of a 490×270×225 mm cage with a central partition bearing a 7×7 cm opening at grid level, a programmer and an event recorder. The two halves of the grid floor could be electrified separately through a shock scrambler.

The animals were given daily shuttle-box avoidance sessions of 30 trials each. The conditioned stimulus (CS) was a 3 candle power light, the unconditioned stimulus (US) 1.5 mA foot shock; CS-US interval 5 sec; CS and US terminated by an escape response; CS–CS interval 30 sec.

The following behavioral items were recorded: (1) an avoidance response if the rat crossed the partition in the shuttle-box within 5 sec of CS presentation; (2) an escape response if the animal crossed after US presentation; (3) an escape failure if the rats remained on the same side of the box for the entire trial. The intertrial crossings were not recorded by the apparatus used.

Passive Avoidance Conditioned Response

The apparatus derived from that described by Jarvik and Kopp [12] consisted of two compartments with grid floors which could be electrified separately. The first compartment was a 250×250×330 mm Plexiglas box illuminated by a 100 W lamp suspended 50 cm above the box. A guillotine door connected the first with the second 500×250×330 mm dark compartment whose walls were painted black.

The rats were trained in a one trial passive avoidance task. The retest was carried out 30 min after training. The rat was placed in the illuminated Plexiglas box. After 1 min the guillotine door was opened and the latency between the door opening and the entrance into the dark box was measured. When the rat walked into the dark box it received a 1.5 mA foot shock. The trial was terminated when the rat ran back into the illuminated compartment, from which it was then removed. The rat was allowed to remain up to 120 sec in the illuminated compartment without walking into the dark compartment before being removed. Better performance was indicated by longer retest latencies.

Drugs

Scopolamine hydrobromide (BDH) was injected intraperitoneally 40 min before testing, in a volume never exceeding 0.25 ml.

Analysis of the Data

Statistical significance of changes in ACh output, scopolamine effect on active avoidance and of passive avoidance was assessed by means of Student's *t*-test. For active avoidance response statistical significance was assessed by analysis of variance (ANOVA) and the *t*-test.

RESULTS

Figure 1 shows four examples of lesions of the MFN similar to those made by Wenk *et al.* [20]. The lesions included the ventromedial extremity of the pallidum, sometimes extending to the nucleus preopticus lateralis and the nucleus preopticus magnocellularis. The tractus diagonalis was usually not affected. Because of the irregular and widespread distribution of the different parts of the MFN it was impossible to achieve a complete destruction of all nuclei.

The spontaneous ACh output from the frontoparietal cerebral cortex ipsilateral to the lesion, measured 20 days after the lesion, was only 18% lower than in the sham-operated rats, as shown in Table 1. Nevertheless when scopolamine was administered to the lesioned rats the increase in ACh output induced by this anticholinergic agent [3,19] was much smaller than in control rats, as shown in Fig. 2, which illustrates the dose-effect relationship between scopolamine and the peak increase in ACh output for each dose.

The peak increase occurred between 40 and 60 min after scopolamine administration, and the effect lasted more than 100 min. In the lesioned rats 1 mg/kg IP of scopolamine had no effect on ACh output, and the increase brought about by larger doses was approximately 65% smaller than in control rats.

Figure 3 shows the shuttle-box performance of the sham-operated rats and the lesioned rats during 10 days of training. It can be seen that the sham-operated rats always performed better than the lesioned rats, making more avoidance responses during the daily sessions that the lesioned rats. The two groups showed statistically significant linear regressions: $F(1,498)=51.4$, $p<0.001$ for the sham-operated and $F(1,488)=39.0$, $p<0.001$ for the lesioned rats. The difference between the two group performance evaluated by the *t*-test on each day was statistically significant with $p<0.05$ on day 4 and 7 and $p<0.01$ on all other days.

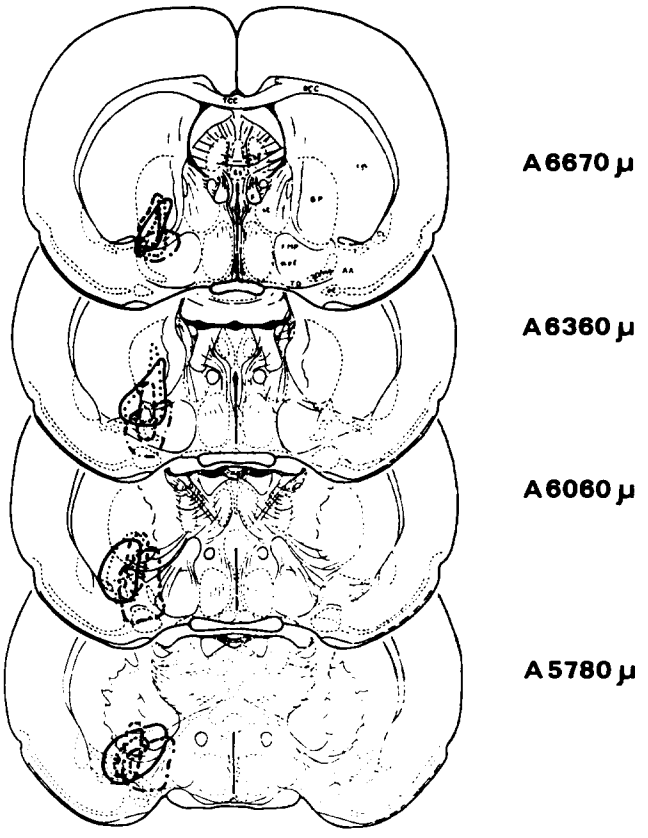

FIG. 1. Schematic drawing of the unilateral lesions of the magnocellular forebrain nuclei (MFN) reproduced from 4 operated rats according to the atlas of Koenig and Klippel [14].

TABLE 1

SPONTANEOUS ACh OUTPUT FROM THE FRONTO-PARIETAL CEREBRAL CORTEX FOLLOWING LESIONS OF THE MAGNOCELLULAR FOREBRAIN NUCLEI (MFN)

Condition	No. Rats	ACh Output ng/min/cm² ±SE	% change	p
Sham operated	7	0.74 ± 0.04	—	—
Lesioned	8	0.61 ± 0.03	−18	<0.01

Three samples, one every 20 min, were collected and bioassayed separately in each rat.

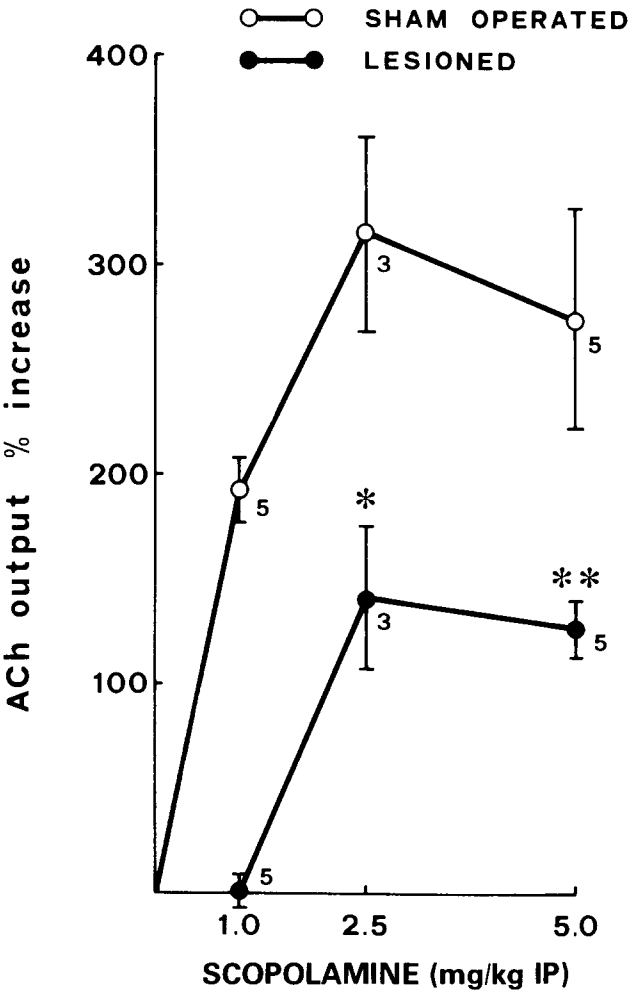

FIG. 2. Maximum percent increase in ACh output after scopolamine administration in sham-operated and lesioned rats. The numbers refer to the rats used for each point. Significantly different from sham-operated: *$p<0.001$, **$p<0.02$.

During the shuttle-box training, if the rats did not respond with an avoidance they usually made an escape response. A small number of escape failures were also observed in both groups. The escape failures were 2.4±0.7 on the first day of training and 1.0±0.6 in the tenth in the sham-operated rats and 4.08±0.6 and 1.6±0.6 respectively in the rats with the MFN lesions. The differences between the two groups evaluated by the t-test were statistically significant ($p<0.01$).

From the histological examination it appears that the lesion of the MFN may sometimes involve also the medial forebrain bundle. A smaller lesion aimed to destroy only the area containing the medial forebrain bundle was therefore placed unilaterally in the brain of 8 rats. The shuttle-box performance of these animals did not differ significantly from that of the sham-operated rats and the number of avoidance responses after 10 days of training was 26.2±2.3.

It is known [5] that small doses of anticholinergic drugs facilitate performance in the shuttle-box. As shown in Fig. 3 the administration of 1 mg/kg of scopolamine from day 3 to day 5 of training significantly increased the number of avoidance responses in the sham-operated rats, but had no effect in the lesioned rats.

In order to evaluate the effect of the MFN lesions not only on acquisition but also on retention of the active avoidance conditioned response, a group of seven rats was trained in the shuttle-box. After eight days of training the number of avoidances had risen from 5.9±1.2 to 24.8±1.9 ($p<0.001$) over 30 trials. The rats were then lesioned and retested eight days later. The number of errors was 22.9±2.6, showing that the lesion had not disrupted the retention of the acquired behavior. This number of avoidance

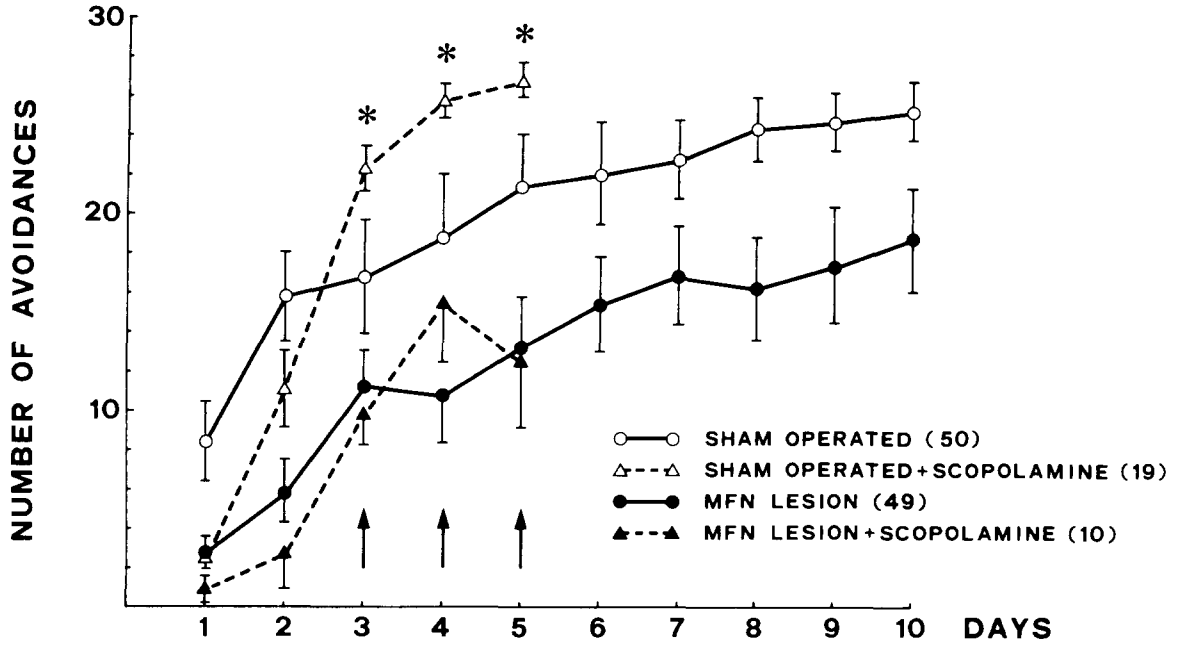

FIG. 3. Shuttle-box performance in sham-operated and in MFN-lesioned rats. In parentheses number of rats. The arrows: scopolamine 1 mg/kg IP. Statistically significant linear regressions for the sham-operated ($p<0.001$) and lesioned ($p<0.001$) rats. Differences between the two groups statistically significant with $p<0.05$ on days 4 and 7 and with $p<0.01$ on all other days. *Statistically significant difference from sham-operated with $p<0.01$.

responses was not significantly different from that found in a comparable group of six sham-operated rats.

The impairment caused by MFN lesions was also evaluated on a passive avoidance conditioned response, by retesting the rats 30 min after the learning trial. The lesion did not affect the initial latency during training. The mean latency was 11.9±2.9 sec in the sham-operated and 11.7±2.3 sec in the lesioned rats. No differences were found in the latency of the scopolamine-treated rats either.

When the rats were retested it was found that the MFN lesions caused a slight but statistically significant impairment in the acquisition of the passive avoidance response, as demonstrated by the shortening in the latency after training shown in Table 2. The impairment was comparable with that brought about by the administration of 1 mg/kg IP of scopolamine 40 min before the training trial. When the same dose of scopolamine was administered to lesioned rats, the passive avoidance conditioned response was strongly impaired; this was revealed by very short post-training latencies.

TABLE 2

EFFECT OF MFN LESIONS AND SCOPOLAMINE ON PASSIVE AVOIDANCE CONDITIONED RESPONSE

Treatment	Dose mg/kg IP	N rats	Retest Latencies (sec) mean±S.E.
Saline	—	38	115.7 ± 3.0
Scopolamine	1.0	51	81.6 ± 6.5*
Scopolamine	5.0	13	66.7 ± 14.9*
Sham-operated	—	12	108.6 ± 9.7
Sham operated + scopolamine	1.0	28	77.1 ± 9.0*
Lesion	—	23	86.3 ± 10.3†‡
Lesion + scopolamine	1.0	20	35.4 ± 12.7*‡

*Significantly different from saline: $p<0.001$.
†Significantly different from saline and sham operated: $p<0.01$.
‡Statistically significant difference between these groups: $p<0.01$.

DISCUSSION

As previously reported [16] we were forced to use rats with only a unilateral lesion of the MFN, since rats with bilateral lesions always died within a week, following a marked loss of weight.

Nevertheless the present experiments show that even a unilateral lesion of the MFN decreases the availability of cortical ACh, and impairs the performance of active and passive avoidance conditioned responses.

No behavioral changes were seen however after a smaller lesion destroying only the area containing the medial forebrain bundle. Similarly [7] the destruction of norepinephrine pathways by local injection of 6-hydroxydopamine in the same area does not impair shuttle-box performance.

It has been demonstrated [17] that 20 days after a unilateral MFN lesion, cholinoacetyltransferase activity still shows a 40% decrease in the ipsilateral cortex, while high affinity choline uptake tends to return to control values. Since high affinity choline uptake can be taken as a measure of the activity of the cholinergic neurons [1,2], the recovery in ipsilateral choline uptake 20 days after lesion could con-

ceivably be due to an increase in metabolic and firing activity of the residual cholinergic neurons [16]. This may explain the small decrease in ACh output at rest in the lesioned rats. However, when ACh output was stimulated by scopolamine, which is believed to remove an inhibitory presynaptic muscarinic control on ACh release [14,19], a marked impairment of cortical ACh output was detected. The increase in ACh output elicited by 1 mg/kg of scopolamine was prevented and that brought about by larger doses showed a 65% reduction. This indicates that a large number of cortical cholinergic nerve endings disappeared following the MFN lesion.

MFN lesions also prevented the stimulatory effect of scopolamine on both output of cortical ACh and active avoidance conditioned response. The possibility of a relationship between the stimulatory effects of scopolamine on both ACh output and shuttle-box performance could therefore be envisaged.

On the other hand the same dose of scopolamine potentiates the disruptive effect of MFN lesions on the passive avoidance conditioned response. It has been demonstrated [9] that scopolamine also potentiates the impairing effect of hippocampal lesions on passive avoidance learning. In that experiment, as in our own, the blocking of muscarinic receptors throughout the brain seems to enhance the effects of the destruction of specific cholinergic pathways, presumably by inhibiting residual cholinergic fibres at postsynaptic levels.

Our experiments confirm that MFN lesions decrease the activity of the cortical cholinergic network and demonstrate its important role in the cognitive functions. They show that even a partial destruction of the cholinergic pathways ascending to the cortex impairs the performance of both active and passive avoidance conditioned responses. However long term memory does not seem to be affected, as shown by the retention of the acquired shuttle-box performance in rats lesioned after training. It must be mentioned in this regard that in our experiments the septo-hippocampal cholinergic pathways, which are also involved in a variety of behaviors [10], are not damaged.

It has been shown [16] that MFN lesions are associated with a reduction in total electrical activity over the lesioned hemisphere; this involves all frequencies, and particularly the high ones. It may therefore be assumed that the cholinergic pathways ascending to the cortex from the MFN are involved in the cortical activation necessary for the acquisition of learned behaviors.

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