

EFFECT OF PHYSOSTIGMINE AND ATROPINE ON THE
SINGLE-TRIAL PASSIVE AVOIDANCE RESPONSE IN RATS

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(Received on March 25, 1982)

Summary : Rats received a single training trial on an inhibitory avoidance (passive avoidance) task and retention trials 24 hr (R-I) and 48 hr (R-II) later, in a special two-chambered apparatus, and the mean step-through latency was determined. The animals were given the drug, either pre-trial, pre-trial plus pre-retention, pre-retention or post-trial. Physostigmine (0.1 mg/kg, ip) and atropine (6.0 mg/kg, ip) had no effect on learning, but they adversely affected retention, particularly at 24 hr. The effect seems to involve central cholinergic mechanisms of retention.

Key words : passive avoidance response
short term memory

physostigmine
atropine

INTRODUCTION

It has been suggested that cholinergic mechanisms (acetylcholine-acetylcholinesterase system) are involved in learning and memory, as a result of which the synaptic conductance is altered (3, 6, 11, 16). The non-specific systems as the limbic and the ascending reticular activating systems also comprise of a powerful cholinergic mechanism and play an important role in memory formation (11). The acquisition of a conditioned response in rats was facilitated by physostigmine (18). The drug has an effect only on the registration phase and has no influence on the consolidation process (15). On the contrary, physostigmine does have an influence on the consolidation of the memory trace (1, 2). Atropine and hyoscine have been found to disrupt the acquisition of active avoidance (13), successive discrimination (17) and passive avoidance learning (1, 2, 4, 5, 8, 12).

We have attempted to elucidate the effect of physostigmine (anticholinesterase) and atropine (cholinoceptor blocker) on the learning and memory process in rats, employing a model which has not been much used so far in such studies.

MATERIALS AND METHODS

Albino rats (Haffkine strain), of either sex, weighing between 150-220 g were used for the study. The doses and pre-treatment times for physostigmine (0.1 mg/kg, ip; 5 min) and atropine (6.0 mg/kg, ip; 20 min) were obtained from published literature (11), and were non-neurotoxic on the rotarod test.

The passive avoidance response (PAR) test: The apparatus was a two-chambered box, comprising a larger bright chamber (30 x 30 cm) exposed to normal room illumination, connected through a hole (diameter, 6 cm) and a guillotine shutter to a smaller dark chamber (15 x 15 cm) that could be electrified by a stimulator (2). Each rat was placed in the bright chamber with its head towards the hole with the shutter open. The rats quickly entered the dark chamber (due to their inherent preference for darkness), where they received a shock (300 V, a.c., 1 sec) once from the grid floor.

The first (R-I) and the second (R-II) retest trials were given 24 hr and 48 hr after the learning session, to assess the extent to which the animal avoided the shock by staying in the bright chamber. The time taken by the animal to enter the small chamber with all its four limbs on the grid floor was called the "STEP-THROUGH LATENCY". At each dose 30 animals were used, and subjected to the trial schedule(s) detailed in Table I.

The drugs employed in this study were physostigmine sulphate (C.H. Boehringer Sohn, Ingelheim, Germany), and atropine sulphate (C.H. Boehringer Sohn, Ingelheim, Germany). The solutions of the drugs were made in 0.9% normal saline and administered intraperitoneally (ip) in a volume of 1 ml/kg body weight.

Statistical analysis of data was done by employing the Student's t-test for unpaired means.

RESULTS

The peak activity time for physostigmine and atropine was 5 min and 20 min respectively. Physostigmine (0.1 mg/kg, ip) significantly impaired the retest latencies at 24 hr in all the groups and at 48 hr, except in Group IV (Table II). Atropine (6.0 mg/kg, ip) only reduced retention in Groups II, III and IV at 24 hr, and in Groups II and IV at 48 hr (Table III).

TABLE I: The grouping of animals and the schedule(s) employed for learning trials and retention testing (I & II) carried out on three consecutive days.

Group	Drug administration (n=6)	Trial schedule		
		Learning (L)	Retention-I (R-I: 24 hr)	Retention-II (R-II: 48 hr)
I	Control	S+Lt+Sh	Lt	Lt
II	Pre-trial	D+Lt+Sh	Lt	Lt
III	Pre-trial + Pre-retention	D+Lt+Sh	D+Lt	Lt
IV	Pre-retention	S+Lt+Sh	D+Lt	Lt
V	Post-trial	Lt+Sh+D	Lt	Lt

S - Saline
Sh - Shock (300 V, a.c., 1 sec)
Lt - Step-through latency
D - Drug

TABLE II: The effect of physostigmine (0.1 mg/kg, ip) on the mean step-through latency of rats in the single-trial passive avoidance response (PAR) test.

Group (n=6)	Learning (L)		Retention-I (R-I)		Retention-II (R-II)	
	Mean±SE(sec)	t@	Mean±SE(sec)	t	Mean±SE(sec)	t
I Control	30.76±4.34	—	72.13±6.43	—	165.33±6.22	—
II Pre-trial	19.05±2.26	2.39 (I/II)	16.25±1.94	8.31*** (I/II)	109.85±5.91	6.46*** (I/II)
III Pre-trial+ Pre-retention	20.64±2.09	2.10 (I/III)	14.80±2.12	8.47*** (I/III)	117.90±7.91	4.71*** (I/III)
IV Pre-retention	27.75±2.54	0.59 (I/IV)	37.65±1.08	5.28*** (I/IV)	159.51±8.80	0.54 (I/IV)
V Post-trial	26.33±2.64	0.87 (I/V)	32.78±2.32	5.75*** (I/V)	129.80±3.25	5.06*** (I/V)

@Student's t test for unpaired means applied: expression () under the t value denotes the groups compared. P Value *** P<0.001

TABLE III : The effect of atropine (6.0 mg/kg, ip) on the mean step-through latency of rats in the single-trial passive avoidance response (PAR) test.

Group (n=6)	Learning (L)		Retention-I (R-I)		Retention-II (R-II)	
	Mean±SE (sec)	t@	Mean±SE (sec)	t	Mean±SE (sec)	t
I Control	33.66±4.04	—	85.48±1.87	—	136.85±2.81	—
II Pre-trial	25.95±3.18	1.50 (I/II)	41.08±2.12	15.70*** (I/II)	104.60±6.77	4.39** (I/II)
III Pre-trial+ Pre-retention	28.91±1.18	1.13 (I/III)	44.03±1.72	16.32*** (I/III)	129.65±4.59	1.33 (I/III)
IV Pre-retention	35.58±2.22	0.42 (I/IV)	38.52±6.65	6.80*** (I/IV)	108.75±9.64	2.79* (I/IV)
V Post-trial	35.58±2.02	0.16 (I/V)	79.21±5.26	1.14 (I/V)	126.85±6.13	1.48 (I/V)

@Student's t test for unpaired means applied ;
expression () under the t value denotes the groups compared.

P Value

* P<0.05

** P<0.01

*** P<0.001

DISCUSSION

Many different types of tasks and training procedures like mazes, discrimination tasks, active and passive avoidance tasks have been used in learning and memory studies. We have selected the single-trial passive avoidance response for this study because the memory of the shock persisted following a single experience, and this learning procedure provided well-marked quantitative indices. This procedure took advantage of the natural instinct of the rodent to avoid light in favour of darkness, and the technique was simple and convenient.

The effect of physostigmine upon performance during acquisition appear to be both dose- and task- dependent (9, 15, 17). Whitehouse (18) reported facilitatory effects of physostigmine (0.025 - 0.075 mg/kg, ip) in the acquisition of an appetitive T-maze discrimination response, whereas, identical doses were reported to disrupt acquisition of a two way avoidance response (15), and passive avoidance response (1, 2, 3).

In our study physostigmine significantly impaired the retention though learning was not significantly affected. Deutsch and Lutzky (7) reported that a given dose of physostigmine can facilitate retention of a poorly learned habit, while impairing retention of a well-learned habit. Rosecrans *et al.* (14) concluded that most of the behavioural effects of physostigmine are related to central acetylcholine-induced inhibition. Increase of acetylcholine to an optimal level facilitated synaptic transmission. Later, the synapses would be blocked as a consequence of acetylcholine excess (6, 11, 16).

Passive avoidance is effectively impaired in rats by pretreatment with antimuscarine drugs like atropine and hyoscine (1, 4, 10, 12), suggesting that the control of responding in passive avoidance situations is cholinergically mediated. The observed effect of atropine could probably be due to an interruption in recent memory, as has been observed by other workers (4, 13). Atropine, by reducing the efficacy of acetylcholine, possibly blocks the transmission to a greater extent at synapses where the concentration of this transmitter is low (4). It has been reported that, in the presence of a cholinergic blockade, the memory trace could be stored in non-cholinergic systems (4). Thus, atropine reasonably interrupts the retention of passive avoidance behaviour, and the manifest effect is possibly guided by an optimal acetylcholine-acetylcholinesterase balancing system. In short, our study suggested that both drugs, physostigmine and atropine, did not affect learning, but affected retention.

ACKNOWLEDGEMENTS

One of the authors (N.J.) is thankful to the University of Rajasthan, Jaipur for permission to publish this work, and to the Principal, S.M.S. Medical College, Jaipur for providing the necessary facilities.

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ACKNOWLEDGMENTS

One of the authors (M.L.) is thankful to the University of Rajasthan, Jaipur for the award of Ph.D. degree and to the P.T.C. 242, Medical College, Jaipur for providing the necessary facilities.

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