THE INFLUENCE OF SOME PSYCHOTROPIC SUBSTANCES ON CONDITIONING USING AN ACTIVE AVOIDANCE PARADIGM

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Abstract

The reflexes represent the quick, involuntary responses of the organism to a stimulus. The mechanisms of both innate and acquired reflex involve the glutamatergic and the dopaminergic transmissions. The aim of this research was to investigate the effect of haloperidol and monosodium glutamate on the formation of conditioned reflex in an active avoidance test, using Wistar rats. Monosodium glutamate(E621, MSG), the sodium salt of glutamic acid, is regulated in the EU as a flavour enhancing agent (E 621). Haloperidol is an antipsychotic, blocker of D_2 receptors. The results obtained indicate that the two substances have a negative influence on the formation of conditioned reflexes.

Rezumat

Reflexele reprezintă răspunsul rapid, involuntar al organismului la un stimul. Transmisiile glutamatergică şi dopaminergică sunt implicate în formarea ambelor tipuri de reflexe, condiţionate sau necondiţionate. Scopul acestui studiu a constat în investigarea efectului haloperidolului şi a glutamatului monosodic asupra formării reflexului condiţionat într-un model experimental de evitare activă, utilizând şobolani Wistar. Glutamatul monosodic (E621, MSG), sarea de sodiu a acidului glutamic, este utilizat în UE ca potenţiator de aromă. Haloperidolul este un antipsihotic, antagonist al receptorilor D_2. Rezultatele indică faptul că cele două substanţe au un impact negativ asupra formării reflexelor condiţionate.

Keywords: monosodium glutamate (MSG, E 621), conditioned reflex, active avoidance test.

Introduction

The reflexes represent the quick, involuntary response of the organism to a stimulus. Reflexes are innate (genetically programmed, not acquired, but involuntary) and acquired (conditioned motor responses, developed after birth). Reflexes represent an important neurophysiological mechanism, essential for body adaptation to external or internal changes and for learning [1, 2, 3]. The mechanisms, the relationship with memory formation/learning and the influence of psychotropic agents on conditioned

reflex can be studied using passive or active avoidance tests. The active avoidance test represents a fear-based avoidance test: while a specific stimulus (auditory or visual - conditioning stimuli) is applied, the animal learns to anticipate the aversive stimulus (electric shock - reinforcement stimulus) and to avoid it. Parameters such as the number of avoidances of the electric shock or the latency of response are used as learning and memory evaluation index [4,5]. The complex mechanisms of conditioned reflex involve glutamatergic and dopaminergic transmissions. Glutamic acid is the major excitatory neurotransmitter of the nervous system. The glutamatergic receptors (NMDA, AMPA, mGlu) are involved in neuroplasticity, neurogenesis and other long-term potentiation mechanisms which have been associated with learning and memory formation [6,7,8]. Hippocampal glutamatergic neurons have been shown to modulate memory and learning process [9]. Activation of NMDA receptors was correlated with memory formation in the passive avoidance tasks [10]. Dopaminergic transmission has been implicated in the performance of avoidance behavior and the dopaminergic innervation of medial prefrontal cortex is responsive to stressful stimuli [11]. Furthermore, dopamine and glutamate transmissions are correlated, inhibition of corticostriatal glutamate-release is mediated by D_2 receptor. One hypothesis is that dopamine, acting *via* D2 autoreceptors, could inhibit glutamate release from endings of nigrostriatal neurons [12]. The aim of this study was to investigate the influence of oral administration of food monosodium glutamate and haloperidol on active avoidance learning. Haloperidol is an antipsychotic, blocker of D_2 receptors [13]. Monosodium glutamate (E621, MSG), the sodium salt of glutamic acid, is a food additive used as a flavour enhancer [14]. The results obtained indicate that the two substances have a negative influence on the formation of conditioned reflexes in an active avoidance model.

Materials and Methods

Materials

Monosodium glutamate (MSG = E 621) was purchased from Sigma-Aldrich, haloperidol was obtained from Terapia SA (România). Automatic Reflex Conditioner apparatus (model 7530, Ugo Basile, Italy).

Experimental animals

Male Wistar rats, weighing 175±5g, were purchased from the University of Medicine and Pharmacy "Carol Davila" Bucharest animal facility. The animals were housed in individualized ventilated cage system, water and granulated food *ad libitum*. The temperature was 20 - 22ºC and the relative humidity was maintained at 35-45%. All procedures were carried out in accordance with the Directive 86/609/EEC-24th Nov.1986,

concerning the protection of animals used for experimental and other scientific purposes and approved by the Ethics Committee of the "Carol Davila" University of Medicine and Pharmacy.

Methods

Learning ability

The effect of the substances was investigated in an experimental active avoidance test, using an automatic reflex conditioning apparatus. In specific pre-set conditions, animals were exposed to a conditioning stimulus – sound, followed by a reinforcement (algic) stimulus – electroshock. The ability of the animal to avoid the reinforcement stimulus when applying the conditioning stimulus was evaluated (the animal avoided the reinforcement stimulus by crossing from one compartment of the conditioning apparatus to another). The conditioning parameters are presented in table I.

Table I

Conditioning parameters of the experimental model

No. crt.	Parameter	Value
1.	Trial number of a session (Tr)	30
2.	Time interval beween 2 trials (PD)	20 s
3.	Conditioning stimulus duration (SD)	10 s
4.	Conditioning stimulus intensity (Al)	70Db
5.	Reinforcement stimulus duration (RD)	5 s
6.	Reinforcement stimulus intensity (Si)	1mA
7.	Delay between conditioning stimulus and reinforcement stimulus	0 s

The results obtained were compared to a reference group treated with haloperidol and a control group, treated with distilled water.

Study design

27 male Wistar rats were conditioned in day 0 of the experiment. Based on the results obtained, the animals were distributed into 3 groups of 9 animals each. The groups received for 14 days: G1 (control group)-distilled water, by gavage, 1 mL/100g b.w.; G2-MSG, by gavage, 375 mg/kg b.w., 3,75% solution; G3-haloperidol, intraperitoneal, 0.05 mg/kg b.w., 0.01% aqueous solution. After 14 days of treatment, the rats were conditioned once per day over a 4 day period. The animal response was quantified as it follows: the time interval between the conditioning stimulus and the animal response – latency (La); the number of crossings of the animal during the conditioning stimulus (St); the number of crossings of the animal during the reinforcement stimulus (Re), the number of reinforcement stimuli received integrally (RT).

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA. The

type of distribution of the animal response was established with D'Agostino & Pearson test. The statistical analysis used parametrical t Student test (for normal distribution) or nonparametrical Wilcoxon test (for abnormal distribution). The applied tests have a 90% confidence interval, statistical differences were considered for p value < 0.05. The results are expressed as mean±standard deviation.

Results and Discussion

The evolution of the medium values of the parameters recorded during the conditioning process for all three groups, as well as the percentual change (Δ%) when compared to the initial values, is given in tables II-V. The percentual change when compared to control group is illustrated in figures 1-3.

Table II

Variation of the medium values of latency for the conditioning process

		Latency (seconds)				
Group I (Control) (distilled water 1mL/100g bw)	M± DS	2230,25 ±285,92	2105,13 ±323,96	2134,63 ±503,11	1756,13 ±535,00	1322,38 ±567,99
	Δ% vs. initial		-5,61	-4,29	-21,26	-40,71
	Statistics		ns	ns	p<0.05*	p<0.05**
Group II (MSG 375 mg/kg bw)	M± DS	2335,63 ±448,6	2798,25 ±134,6	2668 ±189,7	2744,5 ±169,8	2097,25 ±418,3
	Δ% vs. initial		19,81	17,71	17,51	-10,21
	Statistics		p<0.05*	p<0.05*	p<0.05*	ns
Group III (Haloperidol 0.05mg/kg bw)	M± DS	2231,75 ±364,5	2355,5 ±436,0	2595,88 ±260,4	2848,75 ±135,2	2812,38 ±119,8
	Δ% vs. initial		5,54	16,32	27,65	26,02
	Statistics		ns	p<0.05*	p<0.05**	p<0.05**
Δ%= (Final value-Initial value)•100/ Initial value						

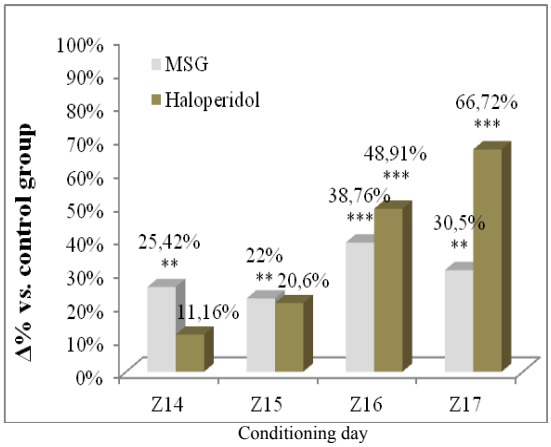

Figure 1

Percentual change (Δ%) of the medium values of latency of groups receiving MSG and haloperidol,when compared to control group for the 4 days of conditioning (day 14, 15, 16, 17). Δ%= Δ % vs. initial value of glutamate group/haloperidol group - Δ% vs. initial value of control group

The latency for the control group decreases progressively during the conditioning period. It is significantly lower than initial in the last two days of testing. For the MSG group, latency increases significantly during the first three days of conditioning but decreases in the last day when compared to initial value. Compared to control group, MSG increases the latency significantly for all testing days. For the haloperidol group, the latency increases significantly for all the testing days when compared to initial value. Compared to control group, haloperidol increases the latency significantly only for the last two days of the conditioning.

Table III

Variation of the medium values of the conditional stimulus for the conditioning process

		Number of crossings during conditioning stimulus (St)				
Group I (Control) (distilled water 1mL/100g bw)	**M± DS**	**1,63** ±1,41	**1,50** ±2,00	**5,13** ±3,18	**8,88** ±8,36	**13,50** ±11,88
	Δ% vs. initial		-7,69	215,38	446,15	730,77
	Statistics		ns	p<0.05*	p<0.05*	p<0.05*
Group II (MSG 375 mg/kg bw)	**M± DS**	**0,875** ±1,58	**0,75** ±1,39	**0,38** ±2,32	**0,75** ±4,17	**4,75** ±5,42
	Δ% vs. initial		-22,22	11,11	111,11	488,89
	Statistics		ns	ns	ns	ns
Group III (Haloperidol 0.05mg/kg bw)	**M± DS**	**1,5** ±1,41	**0,375** ±0,74	**0,25±** 0,71	**0,25** ±0,46	**0,125** ±0,35
	Δ% vs. initial		-75	-83,33	-83,33	-91,67
	Statistics		ns	ns	ns	p<0.05*
Δ%= (Final value-Initial value)•100/ Initial value						

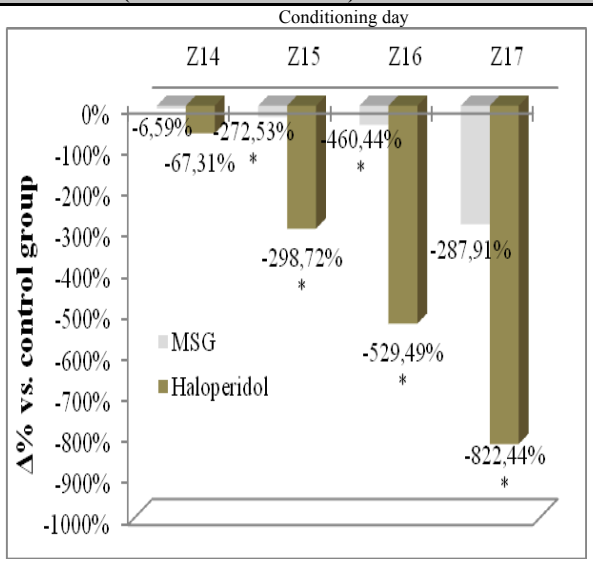

Figure 2.

Percentual change (Δ%) of the medium values of crossings during conditioning stimulus of groups receiving MSG and haloperidol, when compared to control group for the 4 days of conditioning (day 14, 15, 16, 17). *Δ%= Δ % vs. initial value of glutamate group/haloperidol group - Δ% vs.initial value of control group*

St increased for the control group, being statistically significant from the second day of conditioning, thus control group learns quickly that auditive stimulus preceeds an aversive event. St increases for the group receiving MSG, but the increase is not statistically significant when compared to initial. When compared to the control group, the number of crossings is significantly lower for days 2,3 of conditioning for MSG. It is possible that animals treated with glutamate learn that the auditive stimulus preceeds an aversive event, but due to the overstimulation of the central nervous system, the decision of crossing into another room is impaired. For the haloperidol group, St decreases gradually during all conditioning period, which is explained by the inhibition of cognitive processes induced by this drug [16].

Table IV

Variation of the medium values of the reinforcement stimulus for the conditioning process

		Number of crossings during reinforcement stimulus (Re)				
Group I (Control) (distilled water 1mL/100g bw)	**M± DS**	**21,88** ±6,31	**25,13** ±4,32	**20,88** ±3,68	**16,75** ±7,54	**13,00** ±9,37
	Δ% vs. initial		14,86	-4,57	-23,43	-40,57
	Statistics		p<0.05*	ns	ns	ns
Group II (MSG 375 mg/kg bw)	**M± DS**	**18,63** ±8,86	**6,50** ±2,98	**14,75** ±4,74	**16,00** ±4,50	**15,63±**4,56
	Δ% vs. initial		-65,10	-20,81	-14,09	-18,12
	Statistics		p<0.05**	ns	ns	ns
Group III (Haloperidol 0.05mg/kg bw)	**M± DS**	**20,75±** 8,05	**19,63±** 10,36	**13,88±** 8,76	**5,88±** 5,14	**5,00±** 2,27
	Δ% vs. initial		-5,42	-33,13	-71,69	-75,90
	Statistics		ns	p<0.05*	p<0.05**	p<0.05**
Δ%= (Final value-Initial value)•100/ Initial value						

Table V

Variation of the medium values of reinforcement stimuli during the conditioning process

		Number reinforcement stimuli received integrally				
Group I (Control) (distilled water 1mL/100g bw)	**M± DS**	**6,50** ±6,80	**3,38** ±4,87	**4,00** ±3,93	**4,38** ±6,82	**3,50** ±5,83
	Δ% vs. initial		-48,08	-38,46	-32,69	-46,15
	Statistics		ns	ns	ns	ns
Group II (MSG 375 mg/kg bw)	**M± DS**	**10,50** ±9,84	**22,75** ±3,62	**14,88** ±4,88	**13,25** ±4,46	**9,63** ±4,31
	Δ% vs. initial		116,67	41,67	26,19	-8,33
	Statistics		p<0.05**	ns	ns	ns
Group III (Haloperidol 0.05mg/kg bw)	**M± DS**	**7,75±** 8,07	**10±** 10,74	**15,875±** 8,69	**23,875±** 5,00	**24,875±** 2,10
	Δ% vs. initial		29,03	104,84	208,06	220,97
	Statistics		ns	p<0.05**	p<0.05**	p<0.05***
Δ%= (Final value-Initial value)•100/ Initial value						

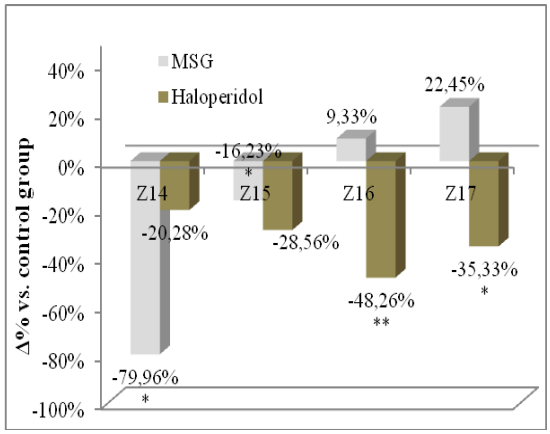

Figure 3

Percentual change (Δ%) of the medium values of crossings during reinforcement stimulus of groups receiving MSG and haloperidol,when compared to control group for the 4 days of conditioning (day 14, 15, 16, 17). Δ%= Δ % vs. initial value of glutamate group/haloperidol group - Δ% vs.initial value of control group

Re decreased when compared to initial for all groups. This decrease is associated with an increase of St and a decrease of totally received stimuli for the groups treated with water or glutamate and with a decrease of St and an increase of totally received stimuli for group receiving haloperidol. Our results are similar with those found in literature for control group, where untreated animals demonstrate learning ability, characterized through progressive decrease of La, increase of St, decrease of Re and of RT [14,15,16]. Literature states that haloperidol impairs the formation of conditioned reflexes with a progressive increase of La, decrease of St, increase of Re and of RT [14,17].

There is no data regarding the influence of MSG orally administered on the formation of conditioned reflex. However, chemical stimulation of hippocampal neurons with glutamic acid in the range 1–10 nmol during learning of an active avoidance response inhibited the acquisition process, increasing the latency time of escape and deteriorating the learning efficiency [8,18].

Conclusions

Rats from the control group learn that inside the conditioning apparatus an auditive stimulus preceeds an electric stimulus. In consequence, the number of stimuli totally received and the latency of response decreased.

For the animals receiving haloperidol, an impairement in conditioning process was noticed, due to the intense inhibition of the central nervous system induced by this neuroleptic.

MSG appears to impair short-term memory, inducing a deficit in acquisition of active avoidance response for the first days of conditioning. The rats treated with glutamate gradually acquired the active avoidance performance after daily repeated training.

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