## MULTIPARAMETRAL ANALYSIS OF PASSIVE AVOIDANCE FORMATION

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*Abstract:* The present research was undertaken for the multiparametral study of passive avoidance behavior conformities in dorsal hippocampus coagulated rats (DHCR) according to special modificated method, which gave possibility, analyzing 19 behavioral parameters, compare passive avoidance reaction's dynamic conformity in different conditions - in presence or absence of habituation (latent learning).

Standard and Step-wise Discriminant Function Analysis by means of Mahalonobis distances and probabilities (P) reveals trustworthiness of betweengroup differences, significance and dynamic of interparametral correlation's coefficients K and consequently gives possibility reveals leading ethological parameters, which determines passive avoidance formation appropriates. The experimental results show, that in DHCR fear emotional reactions are increased. Unexpected electrical stimulation causes dramatically behavioral changes, passive avoidance decrease, but in presence of habituation, preliminary latent learning promote successful passive avoidance reaction. It became evident, that for fulfilling of passive avoidance formation besides the hippocampus crucial role plays latent learning, which corrects operated animals behavioral strategies.

*Key words:* passive avoidance behavior, dorsal Hippocampus, habituation, Discriminant Analysis, correlation.

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The aim of our experiment was the multiparametral study of passive avoidance conformities. Passive avoidance formation was carried out according to our modificated method, which implied using of open field parameters for rat's behavior multiparametral description. Experimental cabin was consisted of 2 large (illuminated, A) and small (dark, B) chambers. Floor of A part was conditionally divided in 4 sectors; Floor of B chamber was electrified (see Fig.1).

Rats tested on passive avoidance formation were divided in 2 groups: Iwith electrolytic coagulation of dorsal hippocampus (DHCR) and II with coagulated neocortex under hippocampus (NCR) (control group). All rats were maintained under identical conditions and surgical procedure was same for each operated group. Stereotaxic coordinates referenced to Bregma were chosen by Paxinos G. and Watson C. (see Brain Atlas in [1]). Electrical coagulation was made by 1.5  $\mu A$  electrical administration for 15 sec.

Experimental animals were given one trial per day for 10 days. They were allowed to move freely for 15 minutes. Test was divided into 60 5-minutes

intervals. Behavioral parameters were fixed in the special compiled protocols, in appropriate columns of which were registered sector's number, which at that time rat was passed. Rats motor activity were measured by number of passed sectors, that gives possibility to evaluate animals activity trajectory.

19 behavioral parameters were registered: latent period of I entrance, sitting duration after I entrance, entry quantity, average entry latent period, summary sitting duration, summary motor activity, short and long-term grooming quantity and duration in different chambers, orientation behavior from and into dark B chamber and accompanying leave-outs and enters quantity, vertical standings quantity, defection and urination.

Such approach gave possibility to evaluate rat's emotional condition during passive avoidance test. For habituation influence evaluation on passive avoidance formation in distinguished groups experiments were carried out in 2 series: I series rats were allowed freely moving possibility between chambers during 5 day. On VI day when rat entered the dark B chamber, separator door was closed and painful irritant through electrified floor was given. After 30 seconds the door was opened and rats again were given freely moving possibility through chambers. II series rats were given painful irritant on first experimental day they entered the B chamber. Such experimental approach gave possibility to compare passive avoidance reaction's dynamic conformity of various groups in different conditions in presence or absence of habituation (latent learning).

Between-group differences were evaluated by Standard and Step-wise Discriminant Function Analysis (see [1]), which by means of Mahalonobis distances and probabilities reveals trustworthiness of between-group differences, significance and dynamic of interparametral correlations coefficients and consequently give measured behavioral parameters integral assessment possibility (see Fig.2).

Step-wise Discriminant Analysis reveals behavioral parameters, high frequently participated in daily analysis, consequently plays crucial role in formation of behavioral strategy:

1. sitting duration after I entrance;

2. average entry latent period;

3. orientation behavior from dark B chamber and accompanying leave-outs quantity;

4. latent period of I entrance.

In presence of 5 -days habituation (I series), in comparison with I-V days, after irritant electrical stimulation in dark B chamber on VI day, into daily Step-wise Discriminant Analysis participate considerable few behavioral parameters, that logically reflects chaotic character of behavioral strategy.

Discriminant Analysis revealed interparametral correlation coefficients **K** for each experimental day and gave possibility to observe their dynamic during the whole experiment. If K < 0, 5, parameters correlated nonsignificantly, if 0, 5 < K < 0, 7, correlation was weak and if K > 0, 7, correlation between

observed behavioral parameters was significant (table 2).

Comparison of correlative behavioral parameters with high frequently participating parameters in Step-wise Discriminant Analysis gives possibility to reveal leading ethological parameters, which determine passive avoidance formation appropriates. Their considerable changes in case of presence or absence of habituation determines different passive avoidance formation conformity in different rat groups.

In both series (I and II), theoretical number of correlated parameters couples were 44, but discriminant Analysis precisely determine their quantity N. It was found, that in I series, under habituation condition correlated parameters couples quantity decreased from 20 to 11 after painful irritation. In nonhabituated rats N=13. We tried to determine frequently correlated couples, playing important role in passive avoidance formation as before and after electrical irritation (I series), as in nonhabituated condition (II series). Such couples were N=6:

1)Entrance quantity – quantity of entrance into B chamber after orientation behavior;

2) Entrance quantity – leave-out of B chamber after orientation behavior from B department;

3) Motor activity – quantity of vertical standings;

4) Motor activity – leave-out of B chamber after orientation behavior from B department;

5) Average entry latent period – motor activity;

6) Average entry latent period – leave-out of B chamber after orientation behavior from B department.

We revealed 2 behavioral parameters, appeared only after electrical irritation in habituated rats: 1) Entrance quantity - average entry latent period; 2) Entrance quantity into B chamber after orientation behavior into B department - leave-outs of B chamber after orientation behavior from B department.

In nonhabituated rats (II series) in addition appeared 5 correlated behavioral couples:

1) Entrance quantity – orientation behavior from B chamber;

2) Orientation behavior from B chamber – accompany leave-outs quantity;

3) Average entry latent period – entrance quantity after orientation behavior into B chamber;

4)Total sitting duration – entrance quantity after orientation behavior into B chamber;

5)Vertical standings quantity – leave-out of B chamber after orientation behavior from B department.



Fig.1. Passive avoidance experimental cabin.



Fig.2. Mahalonobis probabilities dynamic (I series).

		I SE	RIES		II SERIES					
Rat	Intact-NCR		Intact-DHCR		Intact-N	NCR	Intact-DHCR			
Day	Standart	Step- wise	Standart	Step- wise	Standart	Step- wise	Stanzdart	Step- wise		
1	0	0	0	1	0	0	0	1		
2	0	0	1	1	0	0	1	0		
3	0	0	0	1	1	1	0	1		
4	0	0	0	0	0	0	0	0		
5	0	1	0	1	0	1	0	1		
6	0	0	0	0	0	0	0	1		
7	0	0	0	1	0	0	0	1		
8	0	0	0	1	0	1	0	1		
9	0	0	0	1	0	0	0	1		
10	0	0	0	1	0	0	0	1		

Table 1. Between-group differences evaluated by Standard and Stepwise Discriminant Function Analysis. 0 – nonsignificant difference; 1– significant difference.

Correlations (Total)												
	VAR1	VAR2	VAR3	VAR4	VAR5	VAR6	VAR7	VAR8	VAR9	VAR10	VAR11	
VAR1	1	0,09	0,43	0,26	0,56	0,25	0,14	-0,21	0,51	-0,03	0,353	
VAR2	0,09	1	-0,4	-0,54	0,72	-0,53	-0,43	-0,34	-0,37	-0,56	-0,452	
VAR3	0,428	-0,4	1	0,85	0,04	0,96	0,87	0,02	0,89	0,3	0,974	
VAR4	0,265	-0,54	0,85	1	-0,01	0,91	0,88	0,01	0,58	0,24	0,834	
VAR5	0,557	0,72	0,04	-0,01	1	-0,12	-0,11	-0,44	-0,01	-0,52	-0,067	
VAR6	0,247	-0,53	0,96	0,91	-0,12	1	0,94	0,1	0,77	0,3	0,949	
VAR7	0,139	-0,43	0,87	0,88	-0,11	0,94	1	0,08	0,57	0,26	0,84	
VAR8	-0,206	-0,34	0,02	0,01	-0,44	0,1	0,08	1	0,06	-0,12	0,034	
VAR9	0,509	-0,37	0,89	0,58	-0,01	0,77	0,57	0,06	1	0,35	0,872	
VAR10	-0,032	-0,56	0,3	0,24	-0,52	0,3	0,26	-0,12	0,35	1	0,393	
VAR11	0,353	-0,45	0,97	0,83	-0,07	0,95	0,84	0,03	0,87	0,39	1	

Table 2. Sample of correlation coefficients (K) table

(calculated by Discriminant Analysis)

In habituated rats (I series) after electrical irritation was observed disappearance of all correlations connected with orientation behavior and total sitting duration, but correlation between B chambers entrance and leave-out quantity appeared, that is logical appropriateness after painful stimulation. Correlations connected with orientation behavior appeared again in nonhabituated rats (II series). In above mentioned group new important correlation was observed orientation behavior from B dark chamber became correlated with entrance quantity after this orientation behavior. It was found that I (after electrical stimulation) and II series differ by the existence of correlations concerning with orientation behavior. Evidently, electrical irritation in nonhabituated rat exerted violent influence that revealed in intensive orientation behavior with its high correlative pairing.

The obtained experimental results show, that in DHCR fear emotional reactions are increased. At habituation they successful fulfil passive avoidance, but electrical stimulation changes their behavioral scheme in comparison with intact rats. It is evident that DCHR hardly perceive painful irritant as they reveals arduous in perception of incoming fresh information and formation of appropriate behavioral strategy [2,4], but in presence of habituation, preliminary latent learning promote successful passive avoidance reaction. Unexpected electrical stimulation causes dramatically behavioral changes, passive avoidance decrease; rats are not able to avoid painful irritants place on account of chaotical behavior. It became evident, that for fulfill passive avoidance formation besides the hippocampus crucial role plays habituation to novel situation (latent learning) [3], which correct operated animals behavioral strategies. That fact on the basis of multiparametral analysis give rich experimental results for behavioral conformitys neurobiological interpretation.

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