Neurotransmitter reorganization of cognitive functions as the basis of brain adaptation under chronic hypoperfusion. Dopaminergic and cholinergic systems

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Neuroscience problems

An unsolved problem of chronic cerebral hypoperfusion pathology is correction of delayed cognitive dysfunctions. Pharmacotherapy can include the receptor targets of key for cognitive functions neurotransmitter systems.

According to our data, the cholinergic organization of learning and memory changed 6-7 days after permanent occlusion of the common carotid arteries (model 2VO) in the cortex and hippocampus [1]. We assumed that in response to the vascular pathology, the neurotransmitter reorganization concerns with all neurotransmitter systems involved in cognitive functions.It followed that all these neurotransmitter systems should be examined at long-term hypoperfusion times.

Another problem is to find a methodology for analyzing the obtained data, because until now we have not found patterns in hypoperfusion-initiated cholinergic reorganization of functions.

Study goal

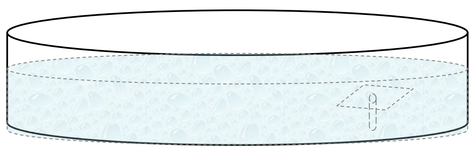
On delayed periods 6-7 days and 1 month of chronic cerebral hypoperfusion (model 2VO), using a statistical method of dividing the data into quartiles, the learning and memory in the spatial contextual model of training in the Morris water maze and the biomarkers of dopaminergic (DA) and cholinergic (AChE) neurons were studding in this functions.

Materials and methods

Out-bred white adult male rats (220-290 g) were used.

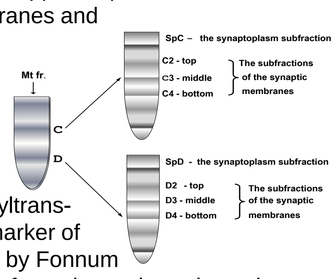
Chronic cerebral hypoperfusion was induced by permanent occlusion of the common carotid arteries by ligation (model 2VO). The sham operated animals were as control group.

Behavior was studied in spatial contextual model of learning and memory in the Morris water maze [2]. Rats training started 6-7 days (7d) or one month (1M) after surgery. The rats were given four daily attempts to find the hidden platform and were trained during two daily sessions. The rats swim time interval for platform achievement (latency time, LT, not more then 60 s) was recorded. The following forms of cognitive functions were observed: the inherited abilities (**1s2**, “rapid one-trial memory”) and the working memory (**1s3-4**) in the first session; the long-term memory on the days after the first session (**2s1** trial); the learning in the second session (**2s2-4** averaged trials).



The apparatus consisted of a circular water pool (diameter, 120 cm; height, 60 cm) filled with water at 24 °C to a depth of 40 cm. A Plexiglas hidden platform (9×9 cm) was submerged 2 cm below the water surface and was placed at the midpoint of one quadrant.

For biochemical analysis rats were used in 2-3 days after the termination training. From fractions of " light" (C) and "heavy" (D) synaptosomes of the cortex and hippocampus were isolated sub-fractions of synaptic membranes and synaptoplaplasm [1]. It allowed to differentiate the cholinergic projective (C) and interneurons (D) of the cortex and hippo-campus.In the sub-synaptic fractions activity of membrane-bound and soluble choline acetyltrans-ferase (ChAT, EC 2.3.1.6), biomarker of the cholinergic (AChE) neurons by Fonnum radiometric method [3], activity of membrane-bound tyrosine hydroxylase (mTr-OH, EC 1.14.18.1), biomarker of dopamine (DA) neurons by Huot and Parent spectrophotometric method [4] was estimated.



Statistical treatment included the correlation analysis of Pearson and a nonparametric Exact method of Fischer. Correlation dependence was considered statistically significant at $p<0.05$. To analyze the results of the study, we used a statistical method of dividing the data into quartiles with upper (highest), middle upper, middle lower and lower (weakest) cognitive abilities to training [5].

Results

I. **Figure 1.** In the control group (sham rats), the rats with different abilities to training in the spatial model of the Morris water maze were represented approximately in the same relationships. In 2VO rats, at the critical time of 7d, the all types of cognitive functions except working memory (1s3-4, red) were disrupted: the animals markedly prevailed in the middle lower and/or lower quartile and were practically absent in the upper quartile). After 1 month 2VO, restoration of functions disturbed after 7 days was observed, but only long-term memory reached the control values. On the contrary, the working (operative) memory was most steady to the common carotid arteries hypoperfusion or its dysfunction was developed more slowly (disturbance of this function was more pronounced at the 1M compared to 7d).

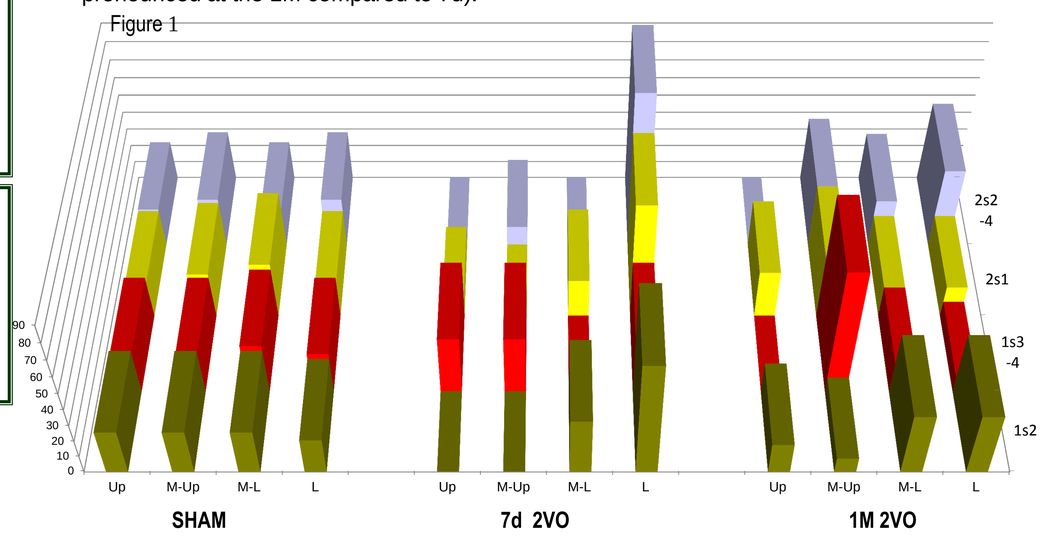
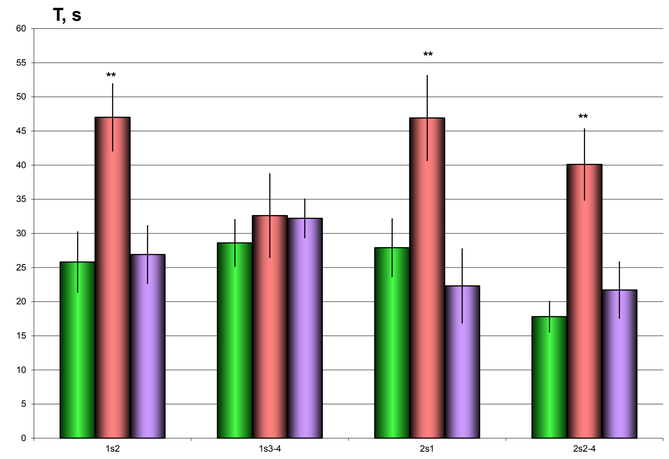


Figure 1. Percent ratios of rats in the quartiles, with different in the spatial model of the Morris water maze in control (Sham, n=17), 7d (n=9) and 1 M (n=11) 2VO groups..
Notation of the cognitive functions: 1s2, rapid one-trial memory, 1s3-4, working memory, 2s1, the long-term memory; 2s2-4, learning in the second session.
Notation of the quartiles of rats with different **abilities to training** in 1s2, 1s3-4, 2s1 and 2s2-4, respectively:
Up, upper quartile (LT≤ 12, 20, 10 and 7 s), n= 4-6 for each type of function (in sham rat group);
M-Up, middle upper quartile (LT= 12 - 19.15, 20 - 34, 10 - 20 and 7 - 12.7 s), n= 4-5;
M-L, middle lower quartile (LT= 19,15 – 37, 34 – 40.5, 20 – 45 and 12,7 – 17,3), n= 4-6 ;
L, lower quartile (LT≥ 37, 40,5, 45 and 17,3), n=4-5.

II. Figure 2. In the mean values, the parameters of the cognitive functions are as follows: **1)** In the sham of rat group (green, n = 17), the values of all functions at the levels of middle (M-Up and M-L) quartiles; **2)** In 7d 2VO rat group, the 1s2, 2s1 and 2s2-4 functions were at the level of L (low) quartile (lilac, $P< 0.01$, n = 9); **3)** In 1M rat group (blue, n = 11), normalization of the 1s2, 2s1 and 2s2-4 functions to control values was; **4)** The function of 1s3-4 in the 7d and 1M 2 VO rats retained the mean control values. Spatial contextual model of learning in the Morris water maze.

Figure 2



III. Figure 3. A comparison of Tr-OH activity and learning and memory measures in the range of quartiles revealed the following: **1)** In the sham rat group, the manifested predominance of negative correlations in Up quartile (green) and the progressive increase in positive correlations in M-Up (light gray), M-L (dark gray) and L (crimson) quartiles (n as in Figure 1); **2)** In the 7d 2VO rat group, the disappearance or inability to evaluate of the DA influences on cognitive functions in all quartiles (n = 0-3 in Up, M-Up and M-L quartiles; n = 1 for 1s3-4 and n = 4-6 for other functions in L quartile); **3)** In the 1M 2VO rat group, there were new DA influences and also the restoration of the old, intact connection (in 2s2-4 in M-Up quartile) (n = 0-3 in Up quartile; n = 1 for 1s2, n = 8 for 1s3-4 and n = 4 for 2s1 and 2s2-4 in M-Up quartile; n = 4 for 1s2 and n = 2-3 for other functions in L quartile).

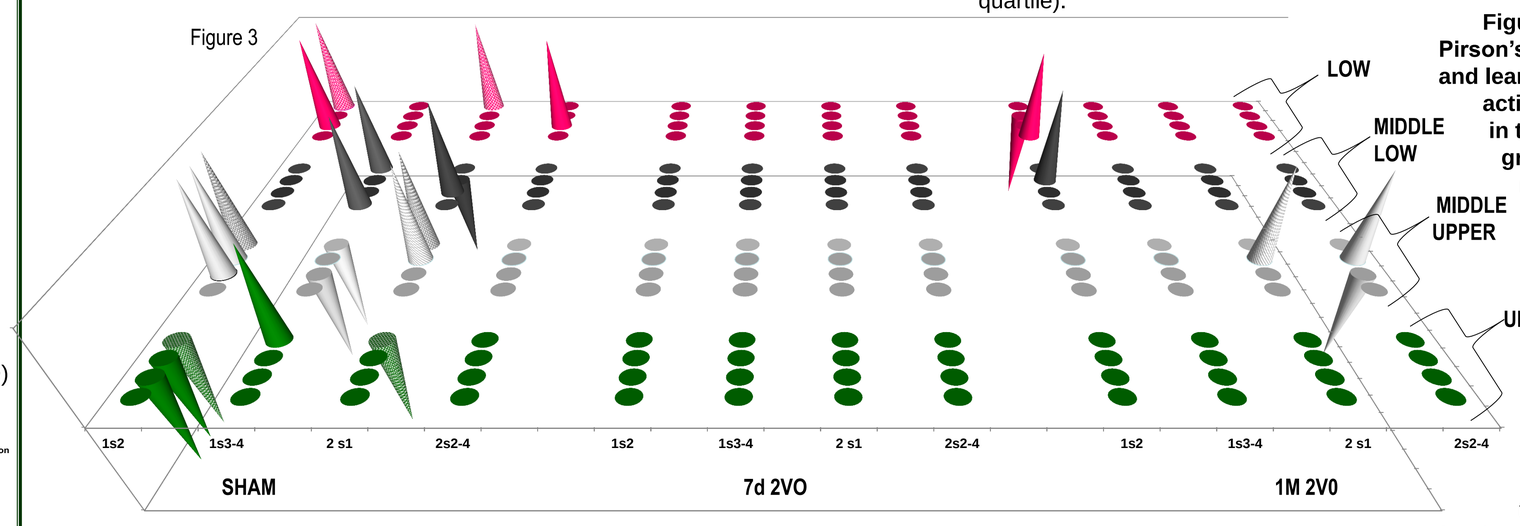


Figure 3. r-Criterion values by Pirson’s test between the memory and learning parameters and Tr-OH activity in the different quartiles in the sham, 7d and 1M 2VO rat groups. Spatial contextual model of learning in the Morris water maze.
In the figure, there are significant r- values only ($p <0.05 - 0.01$) between the indicators as positive (regular pyramids) and negative (inverted pyramids) are shown. In each quartile of each rat group and individually for each function beginning from the front, the membrane sub-fractions of C, D synaptosomes of the cortex and C, D synaptosomes of the hippocampus are consistently located in fours, in which the activity of Tr-OH was measured. Pyramids with a "spheres" pattern in the sham rat group, like sub-fractions (D synaptosomal sub-fraction of the hippocampus), in which the quartile-dependent relationship with the functions (1s2 and 2s1) changed from negative to positive. Pyramids with diagonal hatching in M-Up quartiles (at the intersection of 2s1 - C synaptosomal sub-fraction of the hippocampus), restoration of an intact connection (as in the sham group).

IV. Figure 4. A comparison of ChAT activity in synaptic membranes and synaptoplaplasm sub-fractions and learning and memory measures is presented in Up (green) and L (crimson) quartiles. In the sham rat group, AChE correlations predominated in L quartile. These correlations were predominantly positive and were found both in the cortex and hippocampus.

In Up quartile, AChE influences were detected only in the cortex. Very few rats were in the 7d and 1M groups of Up quartile (n = 0-3), so the AChE communications with the functions were not detected as for DA system.

In L quartile in the 7d time of 2VO, AChE connections completely disappeared with 1s2 (n = 6) and 1s3-4 (n = 3) functions and new AChE connections arose with 2s1 (n = 6) and 2s2-4 (n = 8) functions.

In 1M rat group of L quartile, intact AChE connections with 1s2 function were partially restored (the pyramids with diagonal hatching), and new ones also arose; the positive AChE relations with 2s2-4 in the 7d time of 2VO were converted into negative ones for the 1M of 2VO.

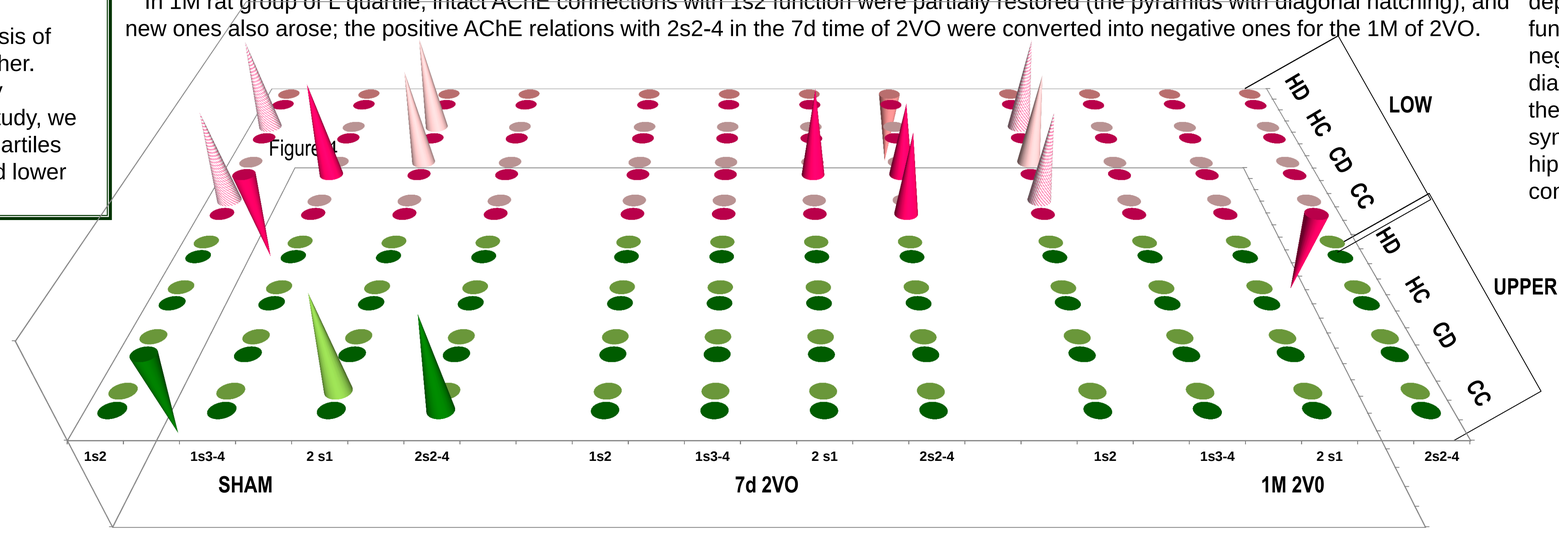


Figure 4. r-Criterion values by Pirson’s test between the memory and learning parameters and ChAT activity in the Upper and Low quartiles in the sham, 7d and 1M 2VO rat groups. Spatial contextual model of learning in the Morris water maze.
As in the figure 3, there are similar significant r- values only ($p <0.05 - 0.01$) between the indicators are shown. In each quartile of each rat group and individually for each function beginning from the front, the sub-fractions of C (CC) and D (CD) synaptosomes of the cortex and C (HC) D (HD) synaptosomes of the hippocampus are consistently located in four pairs in each brain structure, in which the activity of Tr-OH was measured. In each pair, the synaptic membrane sub-fraction (dark color) and then the synaptoplaplasm sub-fraction (light color). Remainder labels as in the figure 3.

References

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Conclusion

The data obtained in the DA system and additional data on the AChE system suggest that the cerebral hypoperfusion causes a strong reduction and reorganization of neurotransmitter links to cognitive functions. Also, our first experience in dividing experimental animals into quartiles made it possible to reveal new results.

1). In sham rat group, in the same sub-fractions, both Tr-OH and ChAT activity have similar values in all quartiles. Therefore, we assume the different DA and AChE organization of learning and memory functions in intact rats from different quartiles. The abundance of positive DA and AChE connections with cognitive functions in rats in the Low quartile does not give a satisfactory result, which deserves attention.

2). In the 7d rat group, Tr-OH activity decreases in all quartiles and in the most sub-fractions (by 30-70%), which agrees with the literature data [6]. ChAT is significantly reduced in rats of the Upper quartile and practically does not change in the rats of the Low quartile. Despite this, the AChE organization of functions varies significantly in both quartiles.

3). Some parallels were seen in the changes in activity of Tr-OH and ChAT (i.e. in the capacity of the corresponding mediator systems) and the abilities for cognitive functions in 1M rat group: 1) the activity of Tr-OH was completely restored in the sub-fraction of C-synaptosomes of the hippocampus and it is sub-fraction, in which an intact DA connection was restored with the function of 2s1 in the rats of the Middle-Upper quartile; 2) on the contrary, the activity of Tr-OH remains low in the sub-fraction of D-synaptosomes of the hippocampus and, possibly, this was due to the absence of the reduction of DA bonds in Tr-OH activity from this sub-fraction.

4) Finally, the very fact of the natural restoration of some DA and AChE links to cognitive functions and the restoration of functions at a remote stage of cerebral hypoperfusion after a period of significant disruption of both functions and the neurotransmitter organization deserve attention.

Thus, the study of neurotransmitter organization and reorganization of cognitive functions is necessary to identify the mechanisms for their regulation and recovery in case of damage and the use of quartiles seems to be fruitful.