Cholinergic Hippocampal Interneurons are critical for Early Spatial Memory Consolidation in Highly Capable Rats and Cholinergic Neocortical Interneurons and Projections are critical in Rats with Less Memory Consolidation Abilities

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INTRODUCTION. Mediator-specific components of functional neuronal networks underlie the study of brain functions in normal and pathological conditions.

In the memory consolidation problem, cholinergic projection systems of the neocortex and hippocampus are intensively studied.

In the neocortex and hippocampus, cholinergic influence comes from two main sources, namely sub-cortical projection neurons and interneurons [1–4]. However, investigation of cholinergic interneurons in cognitive functions is limited [4, 5].

According to our data, in both structures, the presynapses of cholinergic projections and interneurons are concentrated in the fractions of 'light' and 'heavy' synaptosomes, respectively [6, 7].

In our studies, the main tool was the activity of choline acetyltransferase (ChAT), a marker of cholinergic neurons [8]. Moreover, in vivo experiments have shown that ChAT can also indicate the functional state of cholinergic synapses and quantitative changes in the cholinergic synaptic pool [6, 9].

OBJECTIVES. To evaluate the role of both cholinergic projection neurons and interneurons of the neocortex and hippocampus at an early stage of spatial memory consolidation (**2s1**) in normal and chronic brain hypoperfusion conditions (**2VO model**).

AIMS. The normal (control) and 2VO rats were trained with the Morris water maze (MWM), and the activity of membrane-bound and water-soluble ChAT was evaluated in the sub-fractions of light and heavy synaptosomes of the neocortex and hippocampus. For data analysis, the rats were ranked into quartiles according to their performance on stage **2s1**.

METHODS The experiments were carried out in male albino outbred laboratory rats aged 2.5–3.5 months. All animal care and experimental procedures were carried out in accordance with the EU Directive 2010/63/EU.

Chronic Cerebral Hypoperfusion (2VO model) was induced by permanent occlusion of the common carotid arteries by ligation [10] under an appropriate level of Nembutal anaesthesia. Sham-operated animals (control group) underwent the same surgical procedure with the exception of vascular ligation.

MWM test. The MWM was performed following a published standard protocol [11]: one session per day represented four trials of 60 s; the new session started from a new position in relation to the spatially fixed hidden platform; the time to reach the platform (escape latency, **T**) was recorded. **2s1**, first try of the second session, an indicator of early stage of spatial memory consolidation.

The rats were training on days 6–8 (**2VO-7d group**) or days 28–30 (**2VO-1M group**) after the surgery.

Biochemical Analysis involved the methods and procedures of preparation of sub-synaptic fractions from the neocortex and hippocampus and ChAT activity determination in them as described previously [12].

Brain Tissue Preparation. Used the discontinuous sucrose gradients, the light and heavy synaptosomes were obtained from the rough mitochondrial fraction and then the sub-fractions of synaptoplasm and synaptic membranes was extracted sequentially from disrupted synaptosomes. All samples were stored at −80 ◦C until the day of the assay.

ChAT activity was determined by Fonnum's radiometric method [13]. The **sChAT** and **mChAT** activity was measured in the sub-fractions of synaptoplasm and synaptic membranes, respectively.

Statistics. STATISTICA 8.0, Pearson correlation coefficients (***r***), non-parametric Fisher's exact test (FET criterion) and Wilcoxon–Mann–Whitney test (U criterion) was used for statistical analysis. The differences were presented as individual points and the mean ± SEM. The differences and correlations were considered significant at $p < 0.05$.

A note. (1) The results of correlation analysis of **T-ChAT** are presented as follows: the '+' sign of the coefficient ***r*** means that the ChAT activity higher, the T value lower. Accordingly, the sign '-' has the opposite meaning.

(2) The data of three experimental batches performed in different years were compared.

(3) As a rule, correlation analysis in 2VO groups was performed in a combination with data from the control group ('control + 2VO-7d' and 'control + 2VO-1M', respectively). This made it possible to reveal the effect of 2VO on the correlation of the studied parameters in 2VO batches with a small number of variants (n = 1-3).

Quartiles analysis was used according to the ability to **2s1** performance: **1st quartile,** high abilities**; 2nd quartile,** middle-high abilities**; 3rd quartile,** middle-low abilities**; 4th quartile,** low abilities [14].

In norm according to an ability to perform any tested spatial task, laboratory rats acquire stable interquartile boundaries when processing data sets from n ≥ 89 [14]**. This reflects the species, natural properties of the rat for navigation. Therefore, we used these boundaries for quartile distribution of our experimental data.**

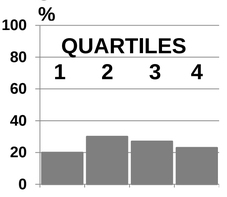
RESULTS

The graphs and schemes indicate the fractions of synaptosomes: CC and CD, light and heavy synaptosomes of the neocortex; HC and HD, light and heavy hippocampal synaptosomes.

CC CD HC HD

In the schemes, the synaptosomes are highlighted in color, in which the T-ChAT correlation was detected, and they indicate the belonging of ChAT (m, mChAT; s, sChAT) and the sign of correlation.

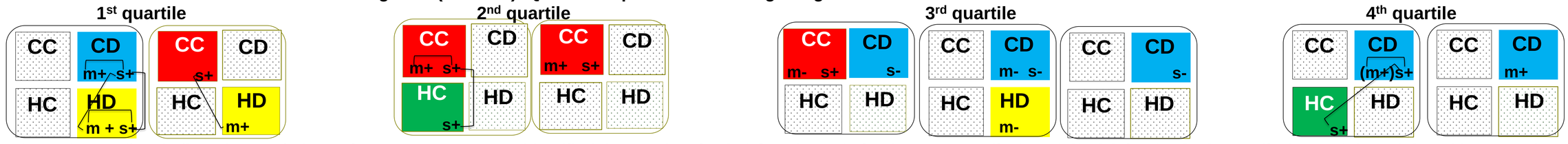
Figure 1



Distribution of rats inquar-tiles based on their ability to **2s1.** The total n = 32.

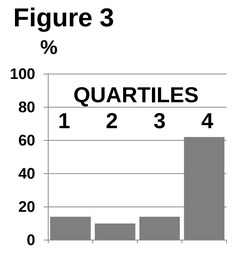
I. CONTROL GROUP

Figure 2 (Scheme). Quartile-dependent cholinergic organization of 2s1 function in the brain of intact rats



In each quartile, cholinergic populations of synaptosomes were identified that are key for 2s1 function: **HD** for the **1st quartile** (n = 4-6), **CC** for the **2nd quartile** (n = 4-11), and **CD** with a negative dependence of T-ChAT for the **3rd quartile** (n = 4-10) and positive T-ChAT for the **4th quartile** (n = 4-8). Other identified populations of synaptosomes could not be involved in the neural network, and this did not affect the quality of the function. At the same time, a positive correlation was generally observed between these fractions and the key ones (square brackets, everywhere *r*+). Apparently, the involvement of these non-key synaptosome populations depended on the individual neuronal organization within the quartile (for more details see [14]).

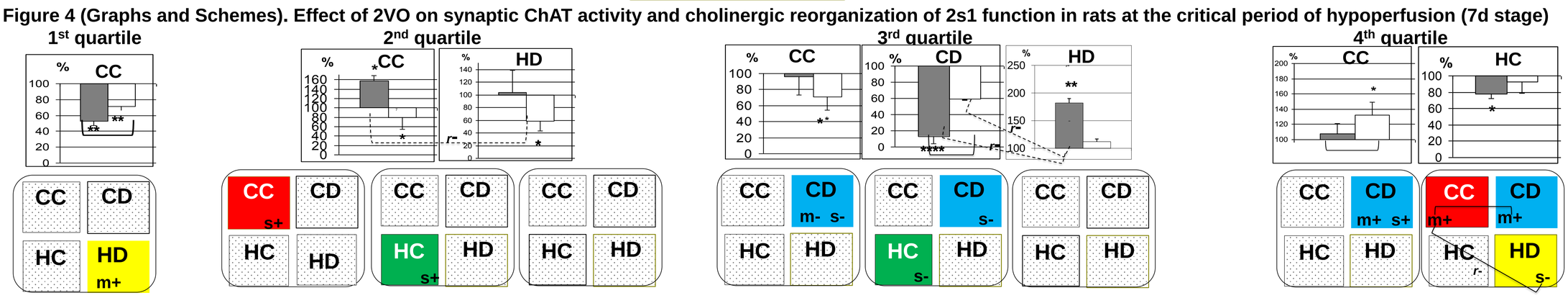
Figure 3



After 6-8 days of 2VO, the ability of rats to early con-solidation of 2s1 was radi-cally impaired. Rats with low ability (**4th quartile, 62%).** The total n = 29.

II. 2VO-7d GROUP

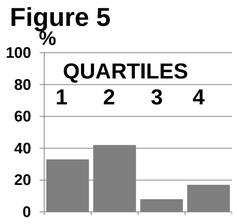
Figure 4 (Graphs and Schemes). Effect of 2VO on synaptic ChAT activity and cholinergic reorganization of 2s1 function in rats at the critical period of hypoperfusion (7d stage)



(**Figure 4)** At the critical stage of hypo-perfusion 7days, 2VO markedly affected the rat cholinergic synaptic pool. We believe that this could be the reason for the deterioration in abilities. **On Graphs**, evidence of a transition to lower quartiles: **(1)** from the **1st quartile** to the **2nd quartile** due to a suppression of sChAT activity in the HD fraction and an activation of mChAT in the CC fraction, which are a stable and negative conjugate (***r-***, square brackets hatched, n=1-3); **(2)** from the **1st quartile** to the **3rd quartile** due to an activation of mChAT in the HD fraction and suppression of mChAT and sChAT activity (degeneration or elimination of the corresponding presynapses) in the CD fraction, which are also a stable and negative conjugated (n = 1-4) ; **(3)** possibly from the **2nd quartile** to the **3rd quartile** due to a suppression of sChAT activity in the CC fraction and (see Schem) the occurrence of a negative T-sChAT correlation. Most of the rats moved into the **4th quartile**. (n = 5-19). Therefore, it was especially difficult to identify a source of cholinergic reorganization. Our assumptions (**Graphs**): **(1)** a conjugated sChAT-mChAT activation in the CC fraction was a summation multidirectional response to 2VO in rats from all quartiles; (2) a decrease in mChAT activity in the HC fraction was a summation response in rats from **2nd** и **4th quartiles**. **// Schemes.** The reorganization of the synaptic pool led to a change in the cholinergic organization of 2s1 in all quartiles. It is also possible that some correlations could be masked and changed due to the complex and variable composition of the 2nd - 4th quartiles from rats with different native organization of function.

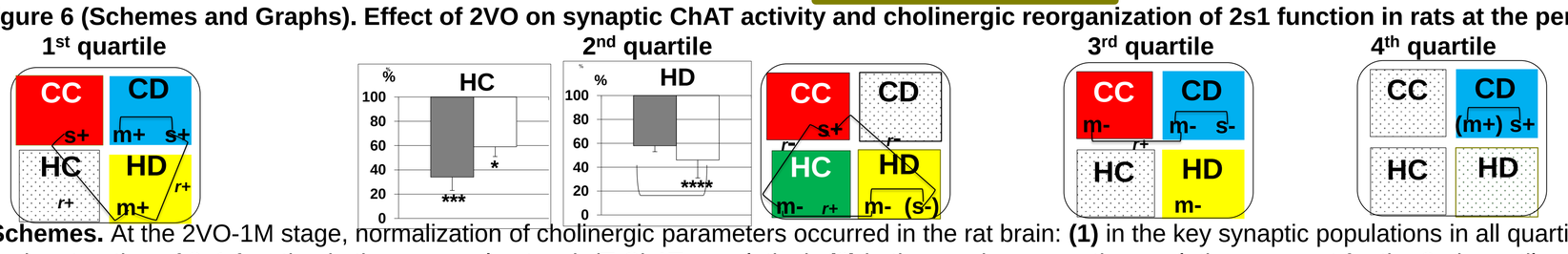
III. 2VO-1M GROUP

Figure 5



After 1 month of 2VO, the ability of rats to early con-solidation of 2s1 was nor-malized in our experimen-tal batch. The total n = 12.

Figure 6 (Schemes and Graphs). Effect of 2VO on synaptic ChAT activity and cholinergic reorganization of 2s1 function in rats at the period of hypoperfusion 1 month (1M stage)



Schemes. At the 2VO-1M stage, normalization of cholinergic parameters occurred in the rat brain: **(1)** in the key synaptic populations in all quartiles and restoration of 2s1 function in the neuronal network (T-ChAT correlation); **(2)** in the non-key synaptic populations, except for the 2nd quartile; **In the 1st quartile** (n = 4), all interstructural connections were restored, as in the intact brain.. **In the 2nd quartile** (n = 5), there was a drop in the m- and sChAT activity, unconjugated in the HC fraction and conjugated in the HD fraction (Graphs); as a result, new, negative T-ChAT associations arose in these fractions and negative correlations with the key CC fraction. **In the 3rd quartile** (n=1), there was a positive association with the key CD fraction, not seen in the intact brain. **Thus,** with the preservation of key fractions at the 2V0-1M stage, the restoration of 2s1 function in the middle quartiles is either not completed or has led to a modification of the neural network.

CONCLUSION

The data of the first quartile analysis in experimental neurobiology of the cholinergic organization of spatial memory consolidation function at an early stage of its formation are presented.

1. The ability to early consolidation of spatial memory depends on the cholinergic organization of the function specific to each quartile.
2. The key cholinergic synaptic populations that determine the ability of rat to spatial consolidation have been identified.
3. High ability to consolidate spatial memory is determined by the inclusion of hippocampal cholinergic interneurons in the neuronal functional network, the synapses of which are concentrated in the HD fraction of the hippocampus.

References

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