

# SPATIAL CONTEXTUAL MEMORY CONSOLIDATION: KEY DOPAMINERGIC AND CHOLINERGIC FUNCTIONAL CONNECTIONS AND THEIR VANISHING UNDER BRAIN HYPO-PERFUSION

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## Study goal

A study of dopaminergic (DA) and cholinergic (ChE) mechanisms of consolidation of spatial contextual memory (SCM) in an intact brain and the reasons for its delayed disturbance under chronic cerebral hypo-perfusion conditions (2VO model).

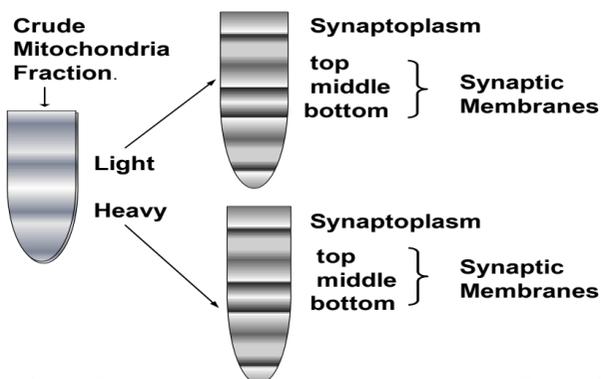
## Materials and Methods

Out-bred white adult male rats were used.

**Chronic cerebral hypo-perfusion** was induced by permanent occlusion of the common carotid arteries by ligation (2VO model). Control group were the sham operated animals **Learning**. A long-term memory was studied in the spatial contextual model of learning in the Morris water maze [DOI:10.1016/0165-0270(84)90007-4]. Rat training started 6-8 days after the surgery. The rats were given four daily attempts to find the hidden platform during two daily sessions. The rats swim time interval for platform achievement (T, latency time) was recorded. A first attempt of the 2nd day of training (2s1 trial) was evaluated as a long-term spatial contextual memory (SCM).

**For biochemical analysis** rats were used in 2 days after the training. From fractions of "light" and "heavy" synaptosomes of the neocortex and hippocampus, the sub-fractions of synaptic membranes and synaptoplasm were isolated [DOI:10.4061/2010/954589]. This allows us to differentiate the sub-synaptic components of the ChE projections from forebrain nuclei (light fractions) and interneurons (heavy fractions) (two main sources of ChE influences in these brain structures [DOI:10.4061/2010/954589]). Functional differences in the ChE presynapses from light and heavy fractions of synaptosomes are usually detected [DOI:10.4061/2010/954589].

### Scheme of Sub-synaptic Fractions Preparation in Discontinuous Gradient of Sucrose Density



In the sub-synaptic fractions, an activity of membrane-bound (m) and soluble (s) choline acetyltransferase (ChAT, EC 2.3.1.6, marker of ChE neurons) [DOI:10.1042/bj1150465] and an activity of membrane-bound (m) tyrosine hydroxylase (mTH, EC 1.14.18.1, marker of DA neurons) [DOI:10.1111/j.1471-4159.2006.04430.x] was determined.

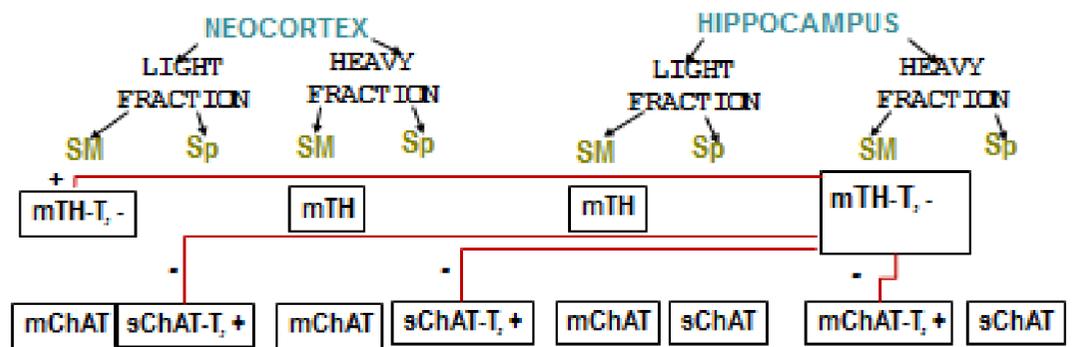
**Statistics.** Pearson Correlation Test and non-parametric Exact Fischer Test.  $P < 0.05$  was statistically significant. To analyze the results of the study, we used a statistical method of dividing the data into quartiles with the rats high and low cognitive abilities to learning and two middle sub-groups between them [DOI:10.1177/0269881115573805jop.sagepub.com]. Each particular method of training memory the limit of ability, and hence the boundaries between quartiles, are constants that are determined by the species. In the spatial learning for rats, the boundaries between the quartiles were stable at  $n = 89$  and  $109$  rats ( $M \pm SEM = 100 \pm 3.7\%$ ) and we used these quartile indicators.

## RESULTS

**I. In the control sub-groups** (sham operated), in rats with high and low capacities, the synaptic correlates of SCM consolidation significantly differed.

In rats with high capacities ( $n = 4$ , **Scheme 1**), the DA presynapses of the heavy fraction of synaptosomes of hippocampus were nodal because they connected all synaptic elements involved in the SCM consolidation (in which TH-T or ChAT-T correlations were revealed).

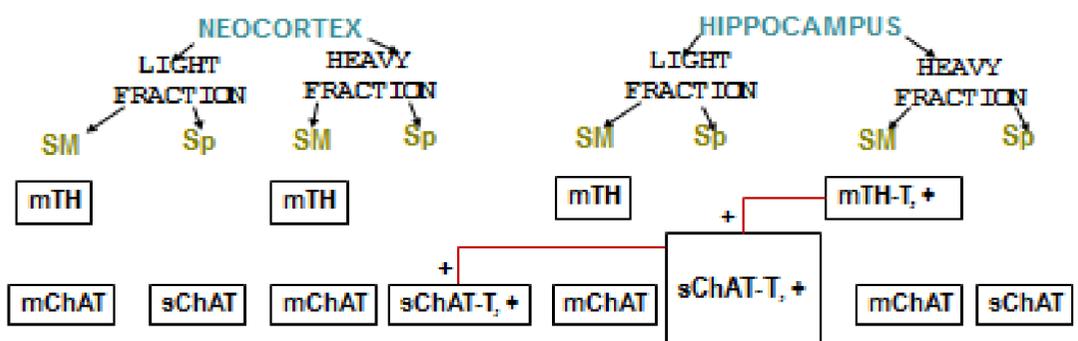
### Scheme 1. Rats with High Capable for SCM Consolidation



Scheme 1. Symbols. SM, synaptic membranes sub-fraction; Sp, synaptoplasm sub-fraction. TH-T or ChAT-T, correlation between the activity of corresponding enzyme and T indicator of SCM consolidation. Red connecting line, correlation between the activity of enzymes from different synaptic populations; +, positive correlation; -, negative correlation. Pearson test.

**II. In rats with low capacities** ( $n = 4$ , **Scheme 2**), ChE presynapses of the light fraction of hippocampus performed a nodal function.

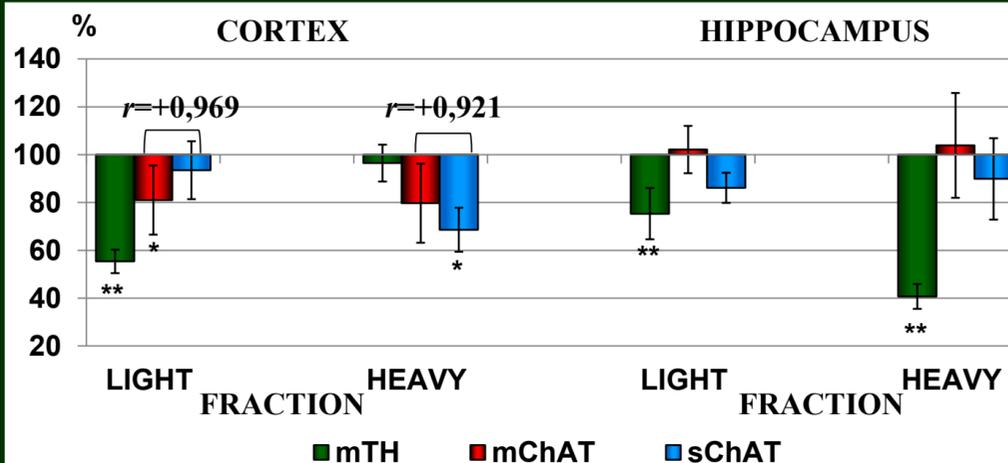
### Scheme 2. Rats with Low Capable for SCM Consolidation



Scheme 2. Symbols as in Scheme 1.

**III. In rats with middle capacities** ( $n = 4$ ), as well as in rats with high capacities, the DA presynapses of heavy synaptosomal fraction of hippocampus were also nodal, but the synaptic links were less extensive and the TH-T correlation was positive in this fraction.

**IV. In 2VO rats** ( $n = 7$ ) at a critical period of 7–9 days of 2VO, the DA link in SCM consolidation disappeared, apparently due to the degeneration of key populations of DA presynapses (**Figure 1**). This was accompanied by an impairment of SCM consolidation, namely: rats with high abilities disappeared and dominated rats with low abilities ( $n = 5$ ). The loss of key ChE synaptic populations from SCM consolidation mechanisms was apparently the result of reorganization of the ChE neural network. Degeneration were observed in the population of small synapses of the light fraction of the neocortex (the decrease in mChAT activity dominated), which was not critical for successful SCM consolidation (critical were presynapses of larger sizes, in which the sChAT-T connection dominates, see Scheme 1). In the 2VO sub-group of rats with low capacities, degeneration of ChE presynapses was observed in the heavy fraction of the neocortex (conjugate decrease in mChAT and sChAT activity). However, the native positive correlation of sChE-T was preserved in this fraction ( $r = 0,896^*$ ) but intersynaptic connections were disturbed.



**Figure 1.** The activity of mTH, mChAT and sChAT after 9–11 days of cerebral hypoperfusion (as a percentage of control).  $r$ , criterion of Pearson test. \*/\*\*,  $P < 0,05/ 0,025$ , Exact Fischer Test.

**Conclusion** In the SCM consolidation mechanisms, certain populations of DA and ChE synapses of the hippocampus and neocortex are organized into a functional neural network according to the nodal principle. High abilities for SCM consolidation are determined by the specificity and extensiveness of intersynaptic connections of DA presynapses of the heavy fraction of hippocampus. Under hypo-perfusion, a loss of DA link led to a loss of ability to maintain the SCM consolidation at a high level.

The synapses of projection ChE and DA systems of the neocortex and hippocampus involved in a formation of memorial trace and this problem is being actively investigated (doi:10.3389/fnsyn.2015.00004, DOI: 10.1038/nature19325, DOI: 10.1111/jnc.14052, DOI: 10.1016/j.cub.2018.09.012). However, the functional properties of ChE and DA neurons, presynapses of which are concentrated in the heavy fraction of hippocampus, have not been identified and practically not studied. At the same time, the participation of these synaptic populations in SCM consolidation deserves attention.