Histone deacetylase inhibitor sodium valproate restores cognitive and olfaction impairments in rats subjected to prenatal hypoxia

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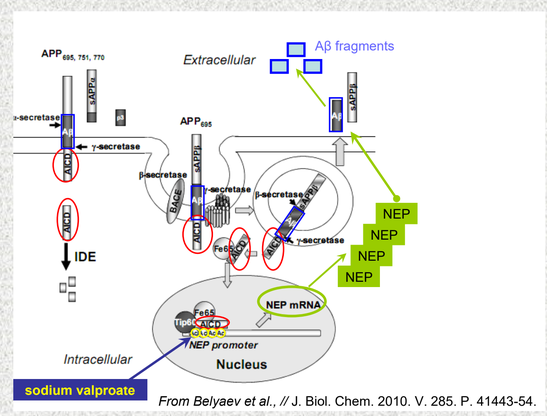
Abstract

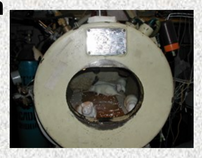
The data accumulated to date indicate that disturbances in the olfactory system and its connections with other parts of the brain are an attribute both of normal aging and early signs of the development of many neurodegenerative diseases. A large number of studies testify to the important role of the olfactory system in the development of dementia and in the pathogenesis of AD. In particular, a number of models have been developed using bulbectomized rodents in which accelerated amyloidogenesis and the development of neurodegeneration take place. In our studies it was shown that impaired embryonic development in rats, as a result of prenatal hypoxia, leads to changes in the normal metabolism of the amyloid peptide and its precursor protein, to alterations of the morphological and functional properties of the nervous tissue of the parietal cortex and hippocampus and to impaired cognitive functions and olfaction. Therefore, prenatal hypoxia in rats can be considered as a zootropic model of the early stages of human neurodegenerative diseases. Investigating the ways to restore the impaired brain functions, we have tested the effects of a histone deacetylase inhibitor sodium valproate which is known to regulate expression of neuronal genes related to neurodegenerative disorders, in particular, of the neuropeptidase neprilysin (the major amyloid-degrading enzyme).



From Belyaev et al., // J. Biol. Chem. 2010. V. 285. P. 41443-54.

Prenatal hypoxia

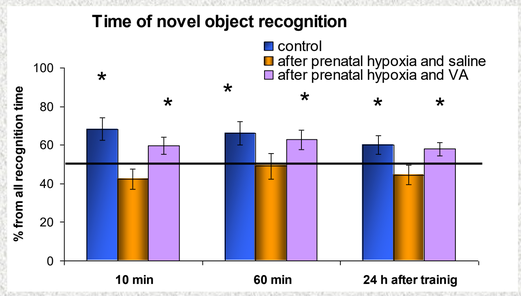
A group of pregnant Wistar rats (200g) on the 14th day of gestation were exposed to normobaric hypoxia in a special chamber with a capacity of 100 liters containing systems of thermoregulation, ventilation, gas analysis and adsorption of exhaled CO_2. During the experiment, the oxygen content in the chamber was reduced from 20.7 to 7.0% and kept at this level for 3 hours. Concentration of carbon dioxide in the chamber did not exceed 0.2%, and the temperature was maintained at 22° C. No more than 10 rats were simultaneously placed in the chamber. Intact control (naïve rats) was taken from the offspring of females not exposed to hypoxia.



Treatment. Sdium valproate (VA) is considered as a tool for up-regulation of neprilysin expression. Rats subjected to prenatal hypoxia were treated by daily *i.p.* injections (n=14) of VA (150 mg/ 1 kg body weight) for two weeks.

Effect of series of *i.p.* injection of sodium valproate on short- and long-term memory (STM and LTM) tested by the novel object recognition test

At the beginning of the test, an experimental animal was placed in a 100x100 cm box with non-transparent 20 cm high walls for 15 min adaptation in the absence of any specific behavioural stimuli. In the first training session, after 2 hours acclimatisation to the experimental arena, the animal was presented with two novel objects (1 and 2) and left to explore them for 10 min. The test was repeated 10 min later to analyse STM and 60 and 24 hours later to analyse LTM. In these tests, which lasted 10 min each, one of the objects (object 2) was changed for a new object (numbered 3 for testing STM, and 4 and 5 for LTM)). Object 1 stayed unchanged in all tests. The time spent to explore each object was recorded by an observer blind to the treatment and expressed as a percentage of the total exploration time computed in seconds. The same scheme of experiments was employed for testing animals from all experimental groups (n=12 for each group). Usually, in this test, animals prefer new objects and spend more time exploring them during subsequent repeat challenges (at 10, 60 min and 24 hours). Absence of statistically significant differences in exploration time between the familiar object 1 and any of the new objects (3, 4 or 5) testifies to the deterioration of memory.
* - p<0.05 - compared to 50% recognition time.



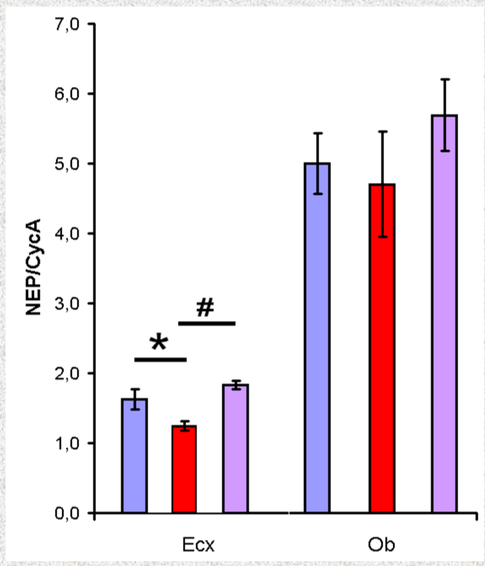
Effect of series *i.p.* injection of sodium valproate on the content of NEP mRNA in the entorhinal cortex (Ecx) and olfactory bulbs (Ob)

Conrol+saline – animals with normal development with saline injection (control)

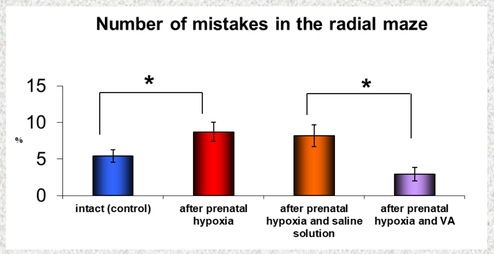
Prenatal hypoxia+saline – descendant from mothers subjected to hypoxia with saline injection

Prenatal hypoxia+VA – descendant from mothers subjected to hypoxia with i.p. injection of sodium valproate

* - differences between control and hypoxic pups (p<0.05)
# - differences between hypoxic pups treated by saline or sodium valproate (p<0.05)

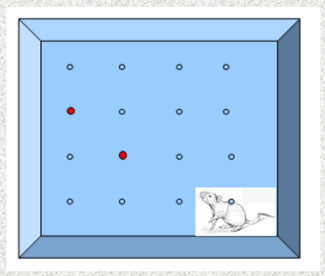


Effect of series *i.p.* injection of sodium valproate on memory in radial maze

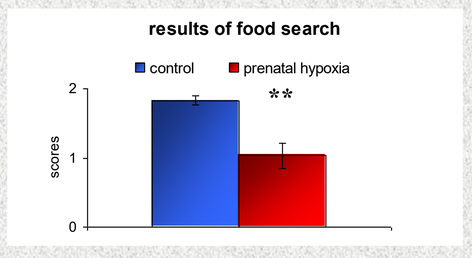


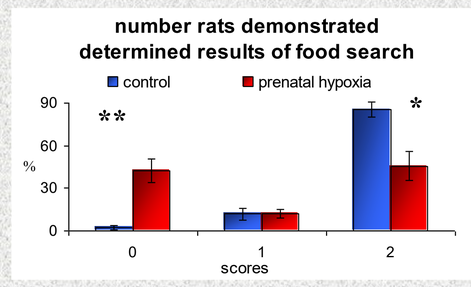
Food search testing

In this paradigm we have used our modification of the test described in (Sun et al., 2016, Frontiers in Neuroscience doi: 10.3389/fnins.2016.00207). Animal testing was performed in a cage with non-transparent walls with the floor dimensions of 100x100 cm and a height of 30 cm. The floor of the chamber had 16 holes of 2 cm diameter. During the experiments scented food pellets (pieces of a cookie of diameter 0.5 cm) were placed at random in two holes 0.5 cm below the floor under the layer of sawdust. In each testing the position of pellets was changed. Before testing the animals had two days of food deprivation. Testing was performed during 15 min (daily for 6 days) and the number of pellets found in each trial estimated as 0, 1 or 2. The bedding in the test cage was changed between the trials. The average number of pellets found by rats in each group has been calculated for 6 days of testing.

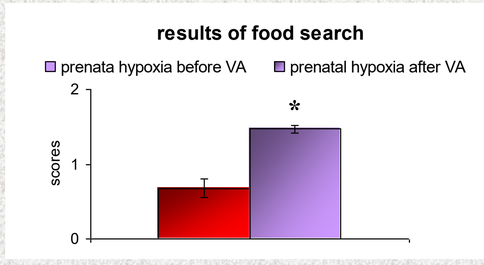


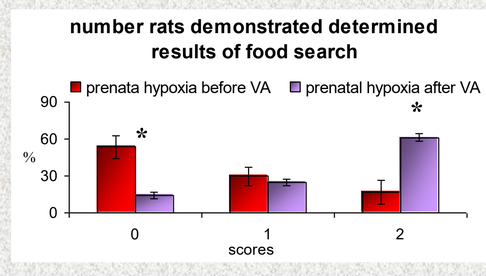
Results of food Search after prenatal hypoxia





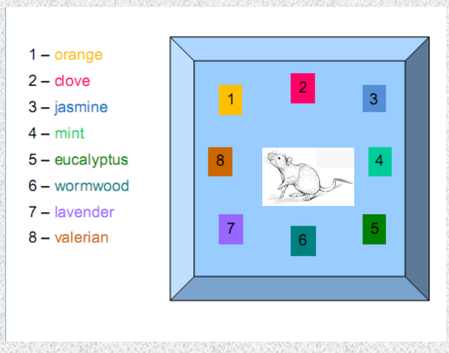
Results of food Search after prenatal hypoxia + VA



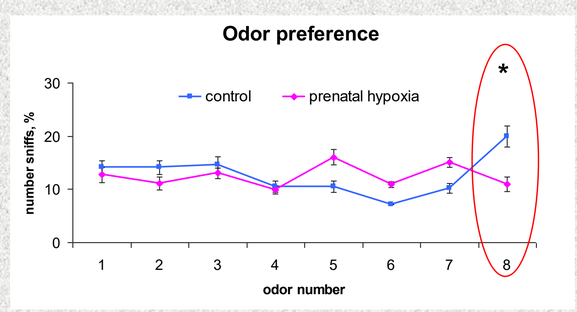


Odor preference

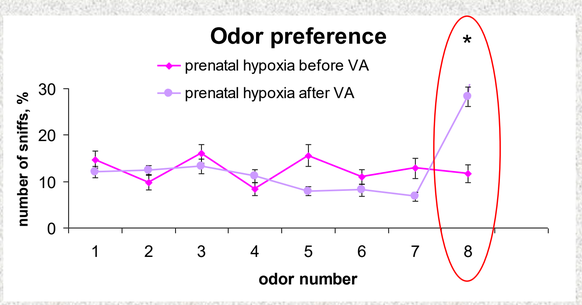
Odour preference paradigm. In this set of experiments rats were offered daily (during 8 days) a battery of 8 vials containing natural oils (orange, clove, jasmine, mint, eucalyptus, wormwood, lavender and valerian) and tested the time spent at each odour during 15 min. In this set of odours only one (valerian) had pheromonal significance for rodents. The position of the vials in each trial was different. The average time spent by rats in each group at each vial during 15 min of the trial have been calculated for 8 days of the testing.



Odor preference after prenatal hypoxia



Odor preference after prenatal hypoxia + VA



Conclusion:

we can conclude that cognitive and behavioral disorders observed in rats after prenatal hypoxia can be attenuated by epigenetic regulation of gene expression, in particular, of neprilysin.

Acknowledgements

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