Supplementary Data

Supplementary Materials and Methods

Experimental protocol for water maze trials

The Morris water maze paradigm was described in details [1, 2]. In brief, a circular swimming tank 80 cm in diameter with a wall height of 40 cm and a hidden platform 5 cm in diameter (State Institute of Biological Instrumentation, RAS, Russia) was filled to a depth of 30 cm with water at 23ºC and rendered opaque by the addition of powdered milk. The tank was mentally divided into four sectors: platform target sector (third), opposite sector (first), adjacent clockwise sector (fourth), and adjacent counterclockwise (second) quadrant. A video monitoring system (TSI, Germany) was used to record the major behavioral parameters in the water-maze paradigm. Initially, all groups of experimental animals were assessed in the water maze to identify any inherent sector preference. None of them showed any preference. To verify that 5XFAD or bulbectomized (OBX) mice did not have motor or visual impairments that may affect the results of memory tests, the latencies required for all these animals to reach the visible platform located in the middle of the third target sector were determined (in three trials) in the Morris water maze before the main spatial training trials initiated in each testing period. The latencies did not differ from the same parameters of control sham-operated (SO) and non-transgenic mice. Mice were then exposed to a total of 20 training trials for 5 days (i.e., four trials per day; 60 s each trial). During each trial, the latency period to locate the hidden platform, submerged at a depth of 0.5 cm so as to be invisible to a swimming animal, was evaluated, up to a maximum of 60 s. Each animal was placed in the water facing the wall of the tank in one of three randomly selected indifferent sectors other than target sector containing the hidden platform. If an animal failed to locate the platform within 60 s, it was placed on the platform for

10 s. Spatial memory was tested on the following day after completion of training with a single probe trial (60 s) in the absence of the hidden platform, beginning from a random position. During the probe trial, occupancy time spent in each sector was recorded.

Differences in behavioral parameters were evaluated with one or two-way ANOVA for repeated measures. Mean latencies and evolution curves for SO, OBX, and 5XFAD (transgenic and non-transgenic) mice to reach the invisible platform were approximately equal. The comparison of SO (n=12), SO+Hsp70 (n=5), OBX (n=10), OBX+Hsp70 (n=6), OBX+ denaturized Hsp70 (n=5), and OBX+ human albumin (n=5) groups did not show any significant group effect in latent periods to reach the invisible platform, neither in the course of the first series ($F_{5,180}$=0.95, p >0.05) nor in the second series, when training and learning tests were performed using other groups of animals at eighth month after bulbectomy (SO (n=9); SO+Hsp70 (n=8); OBX (n=9); OBX+Hsp70 (n=7)] ($F_{3,140}$=1.14, p>0.05). The comparison of these groups of animals did show high significant effect of days of training in the course of the first series ($F_{4, 180}$=38.27, p <0.001) as well as in the second series, ($F_{4,140}$=41.14, p<0.001). One way ANOVA and post hoc analysis revealed significant differences seen on day 1 and 2. On these days, OBX mice found the save platform significantly slower than SO (p<0.05). Analysis of latent time necessary to find the safe platform in the course of 5XFAD transgenic mice learning (Tg+Hsp70 (n=7); Tg (n=7); nTg+Hsp70 (n=7); and nTg (n=7)) showed significant group effect in latent periods to reach the invisible platform ($F_{3,120}$=5,65 p <0.001). The comparison of these groups of animals did show highly significant effect of days of training as well ($F_{4,120}$=16,52 p<0.001). As illustrated by the learning curves depicted in Supplementary Figure 2, all groups improved their performance (i.e., time spent to reach invisible platform) with increased training. *Post hoc* examination revealed no statistical difference in the learning abilities

between any groups over the 5-day training. It is important to mention that the performance of all groups reached approximately the same control levels on the last day (day 5) of the training phase in all experimental series (Supplementary Figure 2A-C).

At 24 h after the last learning trial, mice were examined in a probe trial, in which search patterns were monitored in the absence of a platform. Two-way ANOVA was then performed in order to expose any possible link between groups and sectors as the main factors in the spatial memory test. This analysis of sector occupancy time 1.5 and 8 months after bulbectomy revealed significant effects of sector ($F_{3,148,116}$ =12.5 $p<0.05$ and 17.3 $p<0.001$), but not of group ($F_{5,148}$ = 0.22 and $F_{3,116}$ =1.02 for each $p>0.05$), and there was a significant interaction between group and sector in both periods ($F_{15,148,116}$ = 6.05 and 5.3 $p<0.01$ for each). Eight months following the operation, learning ability and spatial memory were tested in 33 mice. Consequently, a substantial link between sector and group in the water maze test was established using parameters of spatial memory (i.e., occupancy time) in all groups of animals. A group analysis of variance was performed to evaluate the differences in occupancy time in each sector for each group, the results of which are presented in Supplementary Table 1.

Histology and morphology

The cresyl-violet (Nissl) was used for morphological investigation of the state of neurons in temporal cortex and areas of hippocampus: stratum lucidum of area CA3 and stratum radiatum of area CA1. The sections were stained for the Nissl substance with the Cresyl Violet. To do this, the dye-immersed sections were heated in the thermostat (Jouan Quality system, France) at 37°C for 30 min, and after this the sections were placed at 20°C for 6 h. The sections were rinsed in 40% ethanol with subsequent treatment with 70% ethanol containing aniline oil (10:1). The

sections were then dehydrated in ethanol solutions 70%, 96%, and 100%. Finally, the sections were washed in xylene and embedded in Gum Damar (Sigma). Every sixth section of the n. raphe dorsalis, the temporal cortex (the superior and the inferior regions), and the dorsal hippocampus were analyzed with optical microscope (Amplival, Karl Zeiss Jena, Germany) and oculometroscope MOV-15 (LOMO, Russia).

The shape and size of the cells as well as the intensity of staining represent major morphological details taken into considerations in this analysis. Comparative studies of the cellular composition of the temporal cortex and the CA1 and CA3 areas of the hippocampus (1,000 cells for the each structure in the each mouse were counted) were performed using digitizer computer system PDP-12 (Germany). Functional neurons and neurons with distinct pyknotic changes were counted. We distinguished the following categories of neuronal abnormalities: cytolysis, karyolysis, pyknosis, and vacuolization and estimated their frequencies in relation to the quantity of the normal neurons. The pyknomorphic neurons were unusually small with chromatosis, fragmentation, corkscrew circling of the terminals, and conglutination of tigroid blocks. Erosion of the nuclear and nucleolar membranes and hypertrophy of the nucleolus were characteristic of karyolysis. Cytolysis became apparent in the ectopic nuclei with disappearance of the cellular contour. Lysis of the tigroid was also occasionally observed. Vacuolization was evident in alveolar structures and by enlargement of the nucleus and nucleolus and the appearance of confluent lacunae in the cytoplasm. Hsp70 treatment resulted in the dramatic protective effect on morphological state of neurons in all the regions examined. From ten to twelve view fields for each structure were analyzed. We used a 40x objective and a compensative ocular with net magnification of 10. We used an immersion objective of 100x (oil

coefficient of the refraction of 1,565) for investigation the material in more detail. The cell density was determined in 1 mm^2.

Measurement of brain Aβ

The frozen samples of the cortex and hippocampus were thawed and homogenized at a concentration of 4 ml/g in ice cold 2% CHAPS, 20 mM Tris pH 7.7, in the presence of protease inhibitors (10 μg/ml leupeptin, 10 mkg/ml aprotinin and 10 μg/ml AEBSF). The brain homogenates were mixed for 5 min at room temperature. The homogenates were centrifuged at 21,000 x g at 4°C for 30 min. The supernatants from the centrifugation were stored frozen at -80°C, and thawed immediately before use in the ELISA. Aβ levels were determined using an Invitrogen ELISA mouse beta amyloid (1-40) kit which uses a solid phase sandwich ELISA (Invitrogen, Camarillo, CA). In brief, a monoclonal antibody specific for N-terminus of mouse Aβ was pre-coated onto wells of the provided microtiter strips. Samples and standard diluted in the standard diluent buffer were incubated for 2 h at room temperature allowing the Aβ to bind the capture antibody, followed by extensive washing. Samples were incubated with detection rabbit antibody specific for the C-terminus of $Aβ_{1-40}$ for 1 h at room temperature. After washing, samples were incubated with HRP anti-rabbit antibody for 30 min at room temperature. After removal of excess anti-rabbit antibodies, a substrate solution is added, which is acted upon by the bound enzyme to produce color, and after incubation for 30 min at room temperature, the color reaction was stopped. The absorbance was read at 450 nm. The standard provided a linear curve was used to calculate the concentration of Aβ in samples.

Data analysis

All results are reported as means ±S.E.M. Significant differences between mean scores during training trials in the Morris water maze were assessed with two-way repeated measures ANOVA (Statistica 06) with Tukey's post-hoc analysis for multiple comparisons using group and trial day block number during training as well as group and sector of maze as sources of variation. Statistical analysis of the spatial memory training and testing was carried out with two and one-way ANOVA using the program Statistics 6 and ANOVA statistical package (CSS). The p values were reported for repeated measures. The preference for platform target quadrant in comparison with other indifferent quadrants was assessed by post hoc analysis using a multiple-range LSD test. Comparisons the difference of percentage of different kind of pathologies in neurons and level of brain Aβ between experimental groups and were performed using a two-tailed Student's t test. Significance was defined as *p <0.05; ** p<0.01; ***p<0.001.

References

[1] Morris R (1984) Development of a water-maze procedure for studying spatial learning in the rat. *J Neurosci Methods* **11**, 47-60.

[2] Klapdor K, van der Staay FJ (1996) The Morris water-escape task in mice: strain differences and effects of intra-maze contrast and brightness. *Physiol Behav* **60**, 1247-1254.

Figure Legends

Supplementary Figure 1. Localization of Hsp70 labeled with Alexa647 (1-4) and Alexa554 (5, 6) in various brain areas of OBX mice. The images show fluorescently-labeled Hsp70 (red or yellow) in the brain sections of OBX mice 3 h after intranasal injection. Hsp70 is distributed more randomly in cytoplasm of cells than in control NMRI mice (Figure 1) and concentrated in the same brain structures. Photos show its localization in the frontal and temporal cortex (1 and 2), in an area CA3 of the hippocampus (3), in the n. magnocellularis and n. raphe dorsalis (4 and 6), and in the cells of the locus coeruleus (5). It is evident that in most cases Hsp70 has an intracellular localization.

Supplementary Figure 2. Latency to find invisible platform in seconds (mean±SEM) determined during 5 days of training. In OBX mice, training trials in Morris water maze were performed one month (A) or eight months (B) after the operation followed by three weeks of Hsp70 treatment. (C) Training trials were performed for 1 month in two-month old 5XFAD mice and control age groups.

Supplementary Table 1. Factor analysis of sector occupancy time in the water maze for memory tests in two different mouse model of AD-like degeneration: groups of OBX mice and 5XFAD transgenic mice treated with Hsp70 (1.5 and 8 months after bulbectomy and 1 month after Hsp70 treatment in 5XFAD transgenic mice).

Animal groups	Means of Factor occupancy time	
	F	p
First series (1.5 months after bulbectomy)		
SO (n=5)	$F_{3,16}$ =17.481	<0.0001***
SO+Hsp70 (n=4)	$F_{3,12}$=22.671	<0.00001***
OBX (n=6)	$F_{3,20}$=1.64	>0.05 ns
OBX+Hsp70 (n=6)	$F_{3,20}$=22.66	<0.0001***
Second series (8 months after bulbectomy)		
SO (n=9)	$F_{3,32}$ =13.92	<0.001***
SO+Hsp70 (n=8)	$F_{3,28}$=23.29	<0.001***
OBX (n=9)	$F_{3,32}$ =3.5	>0.05 ns
OBX+Hsp70 (n=7)	$F_{3,24}$ =17.82	<0.001***
5XFAD transgenic mice after 1 month Hsp70 treatment (age 3 months)		
nTg (n=10)	$F_{3,36}$ =10.20	<0.001***
nTg+Hsp70 (n=9)	$F_{3,32}$=13.51	<0.001***
Tg (n=6)	$F_{3,20}$=1.36	>0.05 ns
Tg+Hsp70 (n=7)	$F_{3,24}$=6.90	<0.0016**

The data demonstrate that in contrast to SO, SO+Hsp70, and OBX+Hsp70 groups, the OBX animals do not exhibit preference for any of the four sectors in 1.5 month and 8 month periods after bulbectomy. Similarly transgenic (5XFAD) mice in comparison to non-transgenic, nTg+Hsp70 and Tg+Hsp70 could not recognize target sector of Morris water maze. *p<0.05; **p<0.01; ***p<0.001