Introduction: Alzheimer’s disease (AD) is a neurodegenerative disease that causes progressive cognitive and behavioral impairment in the elderly. Okadaic acid (OA), a potent inhibitor of phosphatases 1 and 2A, induces characteristics that resemble AD-like symptoms, including cognitive impairment and hippocampal neurodegeneration. The hippocampus is known to play a major role in long term memory and spatial navigation. Some researchers found that spatial learning impairment was parallel to the magnitude of dorsal hippocampal lesions but not ventral lesions and even a small block of the dorsal hippocampus could support spatial learning in water maze. In the present study, we evaluated and compared effect of intracerebroventricular (ICV) and intrahippocampal bilateral microinjection of OA on spatial memory function and hippocampal structure in rats.

Method and Materials:

Subjects. Experimental protocol was approved by Animal Studies Committee of I. Beritashvili Center of Experimental Biomedicine. A total of 26 male rats. Rats were divided in following groups: Control(icv) - rats injected ICV with aCSF (n=5); Control(hipp) - rats injected intrahippocampally with aCSF (n=5); OAicv - rats injected ICV with OA (n=8); OAhipp - rats injected intrahippocampally with OA (n=8). All data are presented as mean ± standard error of the mean. Differences were considered significant when p < 0.05.

Morris Water Maze. Long-term spatial memory was assessed using a Morris water maze. Briefly, the test was divided into the training phase (day 1) and the retrieval phase 24 h later. Escape platform (10 cm in diameter) was located 2 cm beneath the surface on training day (Fig. 1A). On day 2, rats received eight trials (1-4 trials - block 1; 5-8 trials - block 2), one from each of four equidistantly located start locations (N, S, E, W) in a randomized sequence. The trial ended when the rat climbed on the available platform or until 60 s had elapsed. If a rat could not find the platform after 60 s, it was placed on the platform by the experimenter. Rats were left on the platform for 15 s. Probe tests, during which the platform was removed from the pool, were performed 30s or 24 h after training. The rats were placed in the pool from a novel drop point and allowed to swim for 60 s.

Histology. The surviving pyramidal cells in the hippocampus of rats were visualized by Nissl staining in all behavioral experimental groups. Stained sections were analyzed with fluorescence optic microscope Leica MM AF.

Results: Since there were no significant differences (P>0.05) between Control(icv) and Control(hipp) rats with regard to all behavioral and histological measures these groups were combined into a single one, as of now designated as control (n=10). Nissl staining of hippocampal sections showed that the number of pyramidal cells in the CA1 region of the hippocampus in the control group is significantly higher than that in the OAicv (P<0.001) and OAhipp groups (P=0.01) Fig. 1. The number of pyramidal cells in OAicv group is significantly higher than that in the OAhipp (P<0.01). The Two Way ANOVA for the escape latency showed no significant effect of group (F2,51=0.515, P = 0.601) but showed significant effect of block (F1,52=24.061, P <0.001). There is not a statistically significant interaction between group and block (F2,51=0.111, P = 0.895).

The results of post-hoc (Tukey Test) analysis of differences for escape latency between block 1 and block 2 showed significant difference in control (P = 0.003), in OAicv (P = 0.021) and OAhipp (P = 0.004) treated groups (Fig. 1B) 30s after training, a probe test with the platform removed was performed to assess short-term spatial memory. Short-term spatial memory of the location of the hidden platform is indicated by preference for Sop over Sop. The Two Way ANOVA showed no significant effect of group (F2,51=0.180, P = 0.836) but showed significant effect of sector (F2,51=109.449, P < 0.001); there is not a statistically significant interaction between group and sector (F2,51=0.0379, P = 0.963). The results of post hoc analysis of differences for time spent in Sop and Sop showed significant difference in control (P < 0.001) and in OAicv (P < 0.001) and OAhipp (P < 0.001) treated groups (Fig. 3). 24 hours after training, a probe test with the platform removed was performed to assess long-term spatial memory. Long-term spatial memory of the location of the hidden platform is indicated by preference for Sop over Sop. The Two Way ANOVA showed no significant effect of group (F2,51=0.441, P = 0.646) but showed significant effect of sector (F2,51=13.235, P < 0.001) and interaction between group and sector (F2,51=5.099 P = 0.01). The results of post hoc analysis of differences for time spent in Sop and Sop showed no significant difference in OAicv (P = 0.485) and OAhipp (P = 0.358) treated groups and significant difference in control rats (P < 0.001; Fig. 4). 24 hours after training, during the probe test control rats spent significantly longer than chance (15 s, dotted lines ) in the test sector (Sop) where the hidden platform was located (20.48±1.838, t=0.174, P = 0.867; OAhipp - 14.34±2.178, t=0.299 , P = 0.773).

Conclusions: ICV or hippocampal bilateral microinjection of OA decreased the number of pyramidal neurons in the CA1 and CA3 regions of the hippocampus which is most pronounced in CA1 region. The hippocampal cell loss is lower in the ICV OA injected rats than in hippocampal injected ones. Intracerebroventricular or intrahippocampal bilateral microinjection of OA induced impairment in spatial long-term memory. OA-induced spatial memory impairment may be attributed to the in rat may be considered as a potential animal model for preclinical evaluation of antidementic drug hippocampal cell death. Based on these results OA induced memory deficit and hippocampal cell loss activity.