Homer-1a immediate early gene expression correlates with better cognitive performance in aging

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Introduction

The cognitive and motor function impairment associated with aging is attributed to the progressive accumulation of changes in synaptic structure and function at the molecular level, leading to a loss of biological function. Scaffolding proteins are essential in maintaining synaptic integrity by organizing synaptic proteins. Ves1/Homer are a group of scaffolding proteins that function to convert extracellular signals into intracellular signals at excitatory synapses by organizing signaling proteins at the synaptic site. The Homer-1 gene has four isoforms, one of which is the Ves1-1/Homer-1a short isoform, an Immediate Early Gene product. This short isoform is rapidly and transiently induced by high synaptic activity, while the long Homer-1 isoforms are expressed constitutively.

The long Homer isoforms have a C-terminal coiled-coil (CC) domain that mediates multimerization among Homer monomers, thus regulating protein function. This CC domain is absent in the Homer-1a isoform, therefore making it incapable of multimerizing and allowing it to act as a negative regulator. Homer-1a competes with the long isoforms for binding sites on Homer-1 ligands.

Given the importance of scaffolding proteins in maintaining synaptic integrity, we tested the hypothesis that cognitive performance and learning correlate with altered expression levels of Homer-1a isoform expression.

Materials and Methods

Animals- All animal experiments were approved by the local Institutional Animal Care and Use Committee. Ten young (6 months) and ten old (24 months) male C57BL/6 mice were obtained from the National Institute on Aging and subsequently maintained individually in the institutional vivarium at ambient temperature (23 ± 1°C), under a 12 h light/dark cycle starting at 0600 h. Mice had ad libitum access to food and water except during the testing sessions.

Behavioral Assays- Spatial learning and memory- Spatial learning and memory were measured using a swim maze test. Mice were allowed to swim in a steel tank with a hidden submerged platform (1.5 cm below the surface) provided on a predictable schedule. A computing tracking system recorded path taken and the swimming speed of the animal. Mice were tested for their ability to learn the location of the platform, starting from a different starting point in the tank in each session. Bridge walking- Each mouse was tested for the latency (s) to fall after being placed in one of four bridges, each of which differed in diameter (small vs. large) and shape (round or square), thus providing four levels of difficulty. The maximal latency to fall was set at 60 s. The mean latency to fall was used as measure of performance for each bridge.

Antibodies- The following previously validated primary antibodies were used: goat anti-Homer-1a (1:100; sc-8922) rabbit anti-Homer-1b/c (1:100; sc-20807) rabbit anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 1:5,000; sc-25778). Secondary antibodies were horseshadish peroxidase (HRP) linked donkey-anti-rabbit IgG-HRP (1:10,000; NA954), GE Healthcare) and donkey-anti-goat IgG-HRP (1:5,000; sc-2020).

Quantitative immunoblotting- Animals were euthanized using carbon dioxide asphyxiation. Brains were dissected into forebrain, olfactory bulb and cerebellum and snap frozen in liquid nitrogen. The protein concentration was determined according to the method of Lowry. 25 µg total protein were separated electrophoretically and transferred to polyvinylidene difluoride membranes. Membranes were blocked on a pre-sorbed imaging station. Quantification was performed by densitometry using ImageJ software.

Data was corrected for background and normalized for endogenously GAPDH expression.

Analysis and Statistics- Data were analyzed using Prism 5.01 software. Groups were compared using Student’s t-test. Correlation analysis was performed, calculating a Pearson product-moment correlation coefficient (r) to evaluate the strength of the association. Outlier analysis was performed on values of normalized Homer-1 expression using a difference of two standard deviations from the mean as the exclusion criterion.

Results

Figure 1: Decline in spatial learning and motor coordination with age.

Figure 2: Reduced Homer-1a expression in the aged forebrain.

Figure 3: Homer-1a levels correlate with behavioral measures for decline.

Figure 4: Proposed mechanism of action.

In the young brain, a homeostatic balance between Homer-1a and Homer-1b/c results in a normal equilibrium of synaptic proteins clustered by Homer-1b/c binding and thereby controls intracellular calcium concentrations. In the young mouse, Homer-1a is upregulated in aged animals (P<0.05, ¶ P<0.01 as compared with young animals). Homer-1a expression is decreased by 71% (¶ P<0.01 as compared with young forebrain samples) in aged animals. Data is shown as mean±SEM. Statistical significance is indicated with *P<0.05, ** P<0.01, *** P<0.001 compared with young animals.

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Quantitative immunoblotting was performed on individual normalized quantification of young and aged mice (n=3 per age group). Homer-1a expression was evaluated using the antibody that recognizes only the α isoform of Homer-1a. Homer-1a expression decreased by 71% in aged mice (P<0.05, ¶ P<0.01 as compared with young animals). In aged mice, Homer-1a expression was significantly higher as compared with young animals, while Homer-1b/c expression did not differ between young and aged animals (P=0.67).

Conclusions

• Expression of the Immediate Early Gene product Homer 1a was reduced by 71% in the forebrain of aged mice

• Homer 1b/c did not differ significantly between young and aged mice

• Expression levels of Homer-1a showed a highly statistically significant positive association with the Learning Index as a correlate for spatial learning

• No association was found between Learning Index and Homer 1b/c levels (P=0.67)

This is the first description of a loss of baseline Homer 1a expression levels during aging

We propose a novel cellular mechanism that in the future can be exploited pharmacologically, as supplementing with Homer-1a analogues or mimetics may prevent or delay cognitive decline in healthy aging

Acknowledgements

This study was supported in part by grants AG022550, AG027395 (MJP, NS, PK), and AG010485 from NIH/NIA, RR022570 and RR027093 from NIH/NCRR, the Felix and Carmen Sabates Missouri Endowed Chair in Vision Research and the Vision Research Foundation of Kansas City (P.K.). The authors thank Kathy Vernon and Amy Shah for excellent technical assistance.

The work presented in this abstract has since been published: