

TARGETING NPY Y2 RECEPTOR ACTIVATION IN THE HIPPOCAMPUS VIA ADENO-ASSOCIATED VIRUS SELECTIVELY IMPROVES COGNITIVE FUNCTION IN AGED RATS WITH MEMORY IMPAIRMENT

Ming Teng Koh¹, Rebecca P. Haberman¹, Stacy Foti², Thomas J. McCown², and Michela Gallagher¹

¹Johns Hopkins University, Department of Psychological and Brain Sciences, Baltimore, MD

²University of North Carolina at Chapel Hill, Gene Therapy Center and Department of Psychiatry, Chapel Hill, NC

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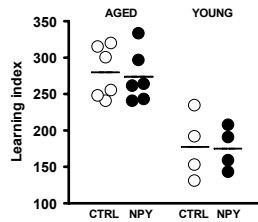
INTRODUCTION

- A condition of medial temporal lobe *hyperactivation* has been reported in fMRI studies of humans with early stages of mild cognitive impairment (Dickerson et al., 2005, *Neurology*, 65:404).
- Similarly, neurons in the CA3 field of the hippocampus are *hyperactive* and fail to encode new information in aged rats with cognitive impairment (Wilson et al., 2005, *J Neurosci*, 25:6877).
- Boosting neuropeptide Y (NPY) neurotransmission, especially through Y2 receptors, has been shown to attenuate hippocampal neuronal excitability in seizure models, indicating a potential application to attenuate hyperactivity associated with neurocognitive aging.
- Here, we examined whether NPY Y2 receptor activation via adeno-associated virus (AAV) in the hippocampus would improve cognitive function in aged rats with memory impairment.

MATERIALS AND METHODS

Behavioral Experiment

Aged (24-mo-old) and young (6-mo-old) Long-Evans male rats were subjected to a standardized assessment of spatial cognition using a water maze (Gallagher, Burwell, & Burchinal, 1993). Aged rats that demonstrated impaired memory performance (i.e., outside the performance range of young controls) and young rats were selected (see side figure) and assigned to either NPY or control treatment. They were injected with either an AAV vector that expresses and constitutively secretes NPY 13-36 (targeting Y2 receptors) or control substances (AAV-GFP or saline) bilaterally into the CA3 of the hippocampus (2 μ l per side) two weeks prior to training in a new water maze environment. Stereotaxic coordinates for the injections were 3.6 mm posterior to bregma, 3.0 mm lateral to midline, and 3.8 mm ventral to the skull surface. Water maze training consisted of 6 trials per day (60 s per trial) for 2 days to locate a submerged escape platform. A probe test was given 24 hr after the last training trial to assess spatial memory.



Viral Vector Construction

AAV-NPY 13-36 vector contained a chicken beta-actin (CBA) promoter driving expression of a fragment of the NPY gene encoding amino acids 13-36 fused to the fibronectin signal sequence (FIB-NPY 13-36). The AAV-GFP control vector contained the CBA promoter driving enhanced green fluorescent protein. AAV2 virus was made using standard procedures at the University of North Carolina Viral Vector Core (Foti et al., 2007). All virus titers were approximately 1×10^{12} particles per ml.

In Situ Hybridization

At the end of the experiment, the brains of the rats were harvested following perfusion. A riboprobe template targeting the FIB NPY 13-36 sequence was generated by PCR from rodent whole hippocampus RNA and modified to contain SP6 and T7 RNA polymerase sites. ³⁵S-UTP labeled riboprobe was generated using the Maxiscript kit (Ambion). Brain sections (40 μ m) from across the entire extent of the hippocampus were hybridized overnight at 60°C in buffer containing the radiolabeled riboprobe. Hybridized sections were then extensively washed, mounted onto slides, and quantified by Phosphorimager system (Molecular Dynamics/Amersham).

NPY Y2 RECEPTOR ACTIVATION IMPROVES MEMORY OF COGNITIVELY-IMPAIRED AGED RATS

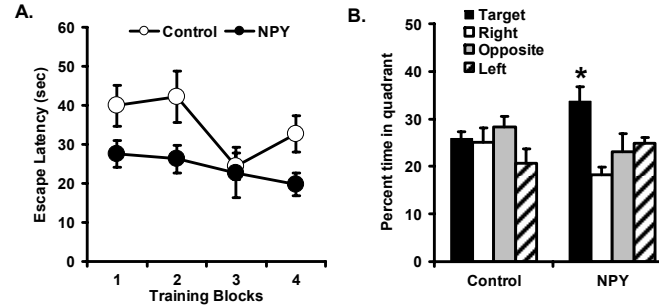


FIGURE 1 (A) Memory-impaired aged rats that received AAV-NPY injection into the hippocampus performed significantly better than their age-matched controls during water maze training, $F(1, 10) = 6.28$, $p = .03$. Escape latencies on the first training trial (data not shown) were not different between the rats in the two groups, but those treated with NPY showed greater proficiency at locating the escape platform than control rats over the course of training.

(B) Aged rats treated with AAV-NPY showed strong memory retention for the escape platform location in a probe trial 24 hr after training as evidenced by a spatial bias for the target quadrant. No such bias was evident in the control group. In addition, rats in the NPY group spent significantly more time in the target quadrant than those in the control group, $t(10) = 2.33$, $p = .04$, suggesting stronger memory in the NPY group.

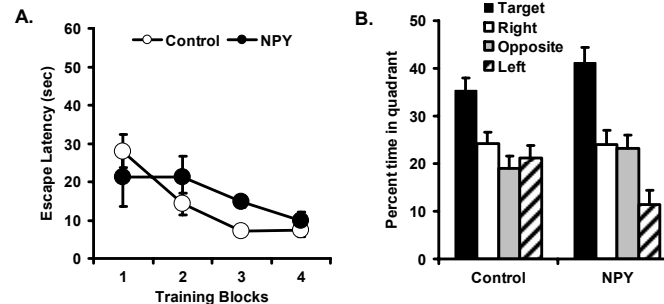


FIGURE 2 (A) In contrast to the results with aged rats, no differences in overall training performance were observed in young rats treated with AAV-NPY or control injections, and (B) no significant differences in retention were apparent between NPY and control-treated young rats as rats in both groups showed good memory for the target location.

mRNA EXPRESSION OF NPY 13-36 IN THE HIPPOCAMPUS

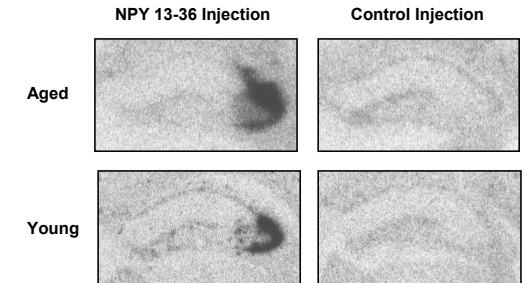


FIGURE 3 Photomicrographs of hippocampal sections of aged and young rats show FIB-NPY 13-36 mRNA was present in the area of AAV injection. The expression levels of the mRNA in the CA3 of aged and young rats were comparable, $p = .67$. Control brains demonstrated no non-specific probe hybridization.

DISCUSSION

- Increased activation of NPY Y2 receptor in the hippocampus via gene therapy improved spatial memory retention in cognitively impaired aged rats.
- NPY is known to regulate excitatory and inhibitory synaptic transmission, and importantly, Y2 receptor activation inhibits glutamate release and reduces neuronal excitability (Vezzani et al., 1999, *Trends Neurosci*, 22:25).
- Treatments that counteract neuronal hyperexcitability such as that demonstrated here may thus be an effective strategy to rescue age-associated cognitive impairment.
- Ongoing studies investigate whether AAV-NPY treatment in aged rats reduces the abnormally high firing rates in the CA3 and restore synaptic plasticity to the hippocampus.

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