Human neural stem cells expressing IGF-1: a novel cellular therapy for Alzheimer's disease

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Background and objective

Alzheimer's disease (AD) is a devastating neurodegenerative disorder that causes age-related dementia in over 5.2 million Americans each year (Fig.1). Currently there is no effective treatment for the disease.

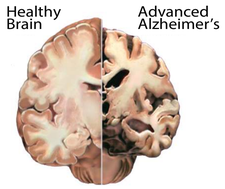


Figure 1. AD patients demonstrate a significant reduction in the size of specific brain regions due to loss of neurons and synapses. Several competing hypotheses exist to describe AD pathogenesis including cholinergic deficiency, abnormal amyloid beta (Aβ) and tau deposits and neuroinflammation. Image adapted from alz.org.

Cellular therapies have the potential to impact AD by multiple mechanisms. Our approach combines two treatment modalities, utilizing neural stem cells (NSCs) not only as a direct cellular therapy, but also as a vehicle to deliver a therapeutic growth factor in order to further protect functional neurocircuitry. Our previous studies show that autocrine IGF-1 production enhances neuroprotective and neurotrophic NSC effects *in vitro* (Fig. 2).

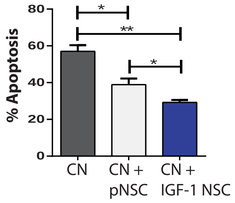


Figure 2. Autocrine IGF-1 provides increased NSC-mediated neuroprotection *in vitro*. Quantification of Aβ-mediated apoptosis (cleaved caspase 3 activation) in cortical neuron (CN) alone or co-cultured with parental unmodified NSCs (pNSC), or IGF-1 producing NSCs. IGF-1 NSCs exhibited an increased neuroprotective capacity compared to pNSCs (*p<0.05 - unpublished data).

Objective: to assess the impact of peri-hippocampal NSC transplantation on memory and learning in the APP/PS1 mouse and to identify the disease mechanisms involved.

Hypothesis: grafted NSCs will improve cognitive impairment and significantly impact disease progression in AD models. We expect multiple mechanisms are involved and that NSCs will establish and support synaptic connections as well as prevent neuronal degeneration by mitigating disease-associated pathologies.

Methods & study design

Methods: Human NSCs (HK532-IGF-1)were derived and provided by Neuralstem Inc. NSC or vehicle (sham) injections were administered to 12 week old male double transgenic (tg) APP/PS1 AD mice (Jackson Laboratories) by three injections into the fimbria fornix bilaterally at 3 sites. Daily immunosuppression was required for the duration of the study (FK506 + mycophenolate). Animals were tested on two hippocampal-dependent behavioral tasks according to the schedule below (Fig. 3). At 28 weeks (16 weeks post-transplant), standard immunohistochemistry (IHC) was used to detect transplanted NSCs (HuNu) and Aβ plaques (6E10 antibody).

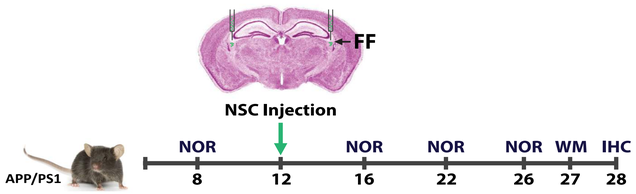


Figure 3. Study Design. Animals were tested on two hippocampal-dependent behavioral tasks: novel object recognition (NOR) and Morris Water Maze (MWM). IHC analyses were performed at the study endpoint at 28 weeks - 16 weeks post-NSC transplant to the fimbria fornices (FF). Image adapted from Blurton-Jones et al. PNAS, 2009.

NSCs improve cognition in AD

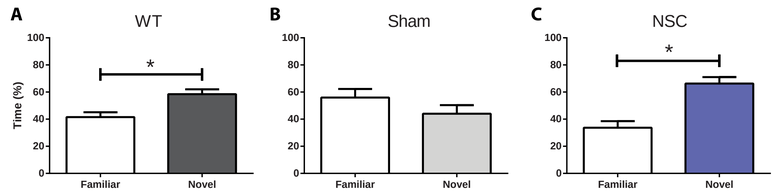


Figure 4. Novel object recognition (NOR). The NOR test was used to assess short-term non-associative memory in non-tg wild type (WT) and APP/PS1 AD mice. WT (A) and NSC-treated (C) mice recognized the novel object and spent significantly more time exploring it (*p<0.05), whereas the sham group were unable to perform the task (B). Pre-op NOR was normal for all animals (data not shown). NOR was repeated at 22 weeks and 26 weeks (data not shown). At both later time points, novel vs. familiar exploration times were not significantly different for the sham group (p=0.432) or the cell group (p=0.056).

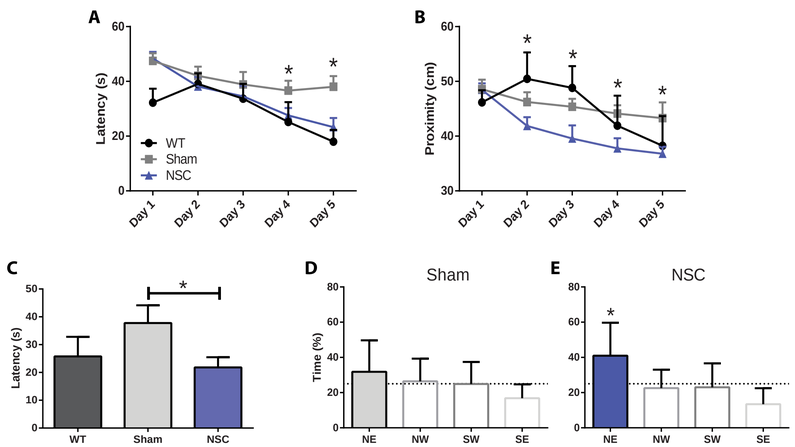


Figure 5. Morris water maze (MWM). MWM was performed 15 wks post-NSC transplant to assess spatial reference and working memory. Hidden platform learning curves over 5 d of training, numbers represent mean ± S.E.M. of daily trials; latency (A) and average proximity (B). NSC-treated mice demonstrated significantly improved performace compared to sham controls during the training period (*p<0.05). In a 24 h probe trial, NSC-treated animals reached the former platform location almost twice as fast as sham controls (C) and spent a significantly increased amount of time in the target quadrant (D), whereas sham-treated AD animals did not (E), demonstrating a strong memory for the platform's former location (*p<0.05 vs. 25%).

NSCs survive and migrate *in vivo*

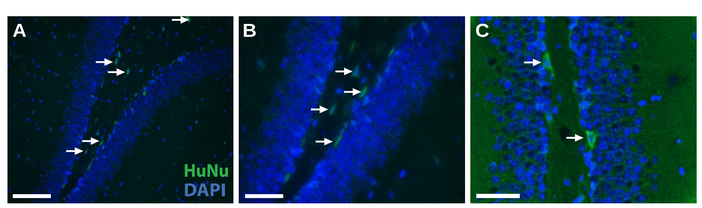
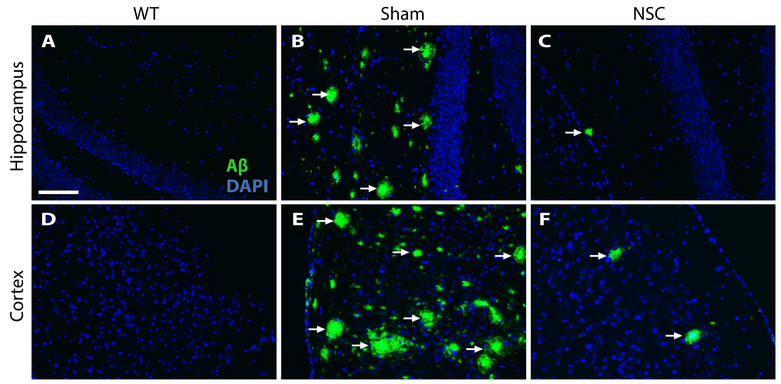


Figure 6. NSCs survive peri-hippocampal transplantation and migrate to the dentate in the APP/PS1 mouse. Representative images of HuNu (green) and DAPI (blue) labeling of human NSCs 16 weeks post-transplantation into the fimbria fornix in the same cohort of animals that completed behavior testing. Transplanted human NSCs (arrowheads) were detected in the dentate gyrus of the hippocamopus demonstrating their ability to migrate from the injection site and survive long term in the AD brain (A, scale bar 100 μm and B, scale bar 50 μm). High magnification image of trasnplanted NSCs at the subgranular polymorph layer of the dentate gyrus (C, scale bar 50 μm).

NSCs reduce Aβ in the cortex and hippocampus



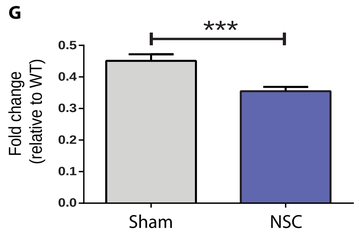


Figure 7. NSCs reduce Aβ load within hippocampal and cortical regions. Representative images of Aβ (green, arrows) in hippocampus and cortex of WT and APP/PS1 AD mice 16 weeks following NSC or vehicle injection (sham) to the fimbria fornix of the hippocampus (scale bar 100 μm). Significant plaque formation was evident in the sham group (arrows, B, E), and was reduced in NSC-treated animals in both hippocampal (C) and cortical brain regions (F). Quantification of fluoresence intensity (fold change relative to non-tg controls) shows a significant reduction in Aβ levels in the NSC group compared to the sham group ***p<0.001, (G).

Summary & future directions

Peri-hippocampal transplantation of human NSCs impacts behavioral and pathological phenotype in the APP/PS1 mouse, supporting further development as an AD therapeutic.

- NSCs enhance cognitive processes involved in learning and memory consolidation
- Benefit is robust as we see short- and long-term effects on cognition
- Transplanted NSCs can migrate from the injection site and survive for up to 16 weeks
- NSCs significantly reduce Aβ pathology in the cortex and hippocampus

Future directions: assess synaptic loss, cholinergic neurons, transplanted NSC differentiation and migration to other sites within the brain; alternative mouse models.

PROGRAM FOR NEUROLOGY RESEARCH & DISCOVERY

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