PRIMER

Exploring the use of neural stem cells to improve cognition in a primer for "Neural stem cells improve cognition via BDNF in a transgenic model of Alzheimer disease"

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ABSTRACT

Alzheimer's disease is the fastest growing cause of dementia, with an estimated number of 131.5 million people to be affected by 2050. Alzheimer's disease is characterized by loss of neurons leading to degeneration of the brain. Memory and cognitive deficits occur in those with Alzheimer's disease. There are two factors that are thought to contribute to the symptoms of Alzheimer's disease: amyloid-β (Aβ) plaques and neurofibrillary tangles made up of tau proteins. Burton-Jones et al. transplanted neural stem cells (NSCs) secreting brain-derived neurotrophic factors (BDNF) into mice and studied the effects. The goal of this primer is to explain how the use of growth factor-secreting NSCs improved cognition in a mouse model of Alzheimer's disease despite not affecting the Aβ plaques and neurofibrillary tangles.

Keywords: Alzheimer's disease, neural stem cells, BDNF

INTRODUCTION

Alzheimer's disease is the leading cause of dementia, with around 46 million currently diagnosed (Bali et al., 2017). Alzheimer's disease is also the fastest growing neurodegenerative disease, as the estimated number of people with Alzheimer's disease will be over 131.5 million in 2050 (Bali et al., 2017). Due to treatment and health care services, Alzheimer's disease has cost the world over a trillion dollars to date, and this number will only increase (Chong et al., 2018). Finding a cure for Alzheimer's disease is important because of the cost, the prevalence, and because there is no effective treatment available that can directly treat the pathologies of Alzheimer's disease.

Oligomers: a polymer whose molecules consist of relatively few repeating units

Unlike other neurodegenerative diseases, Alzheimer's disease has more than one speculated pathology that causes symptoms. There are two main hypotheses for the mechanisms of Alzheimer's disease. One is the amyloid-β (Aβ) plaque hypothesis. Aβ **oligomers** result from the breakdown of amyloid precursor protein (APP). APP is a single-span transmembrane protein, but its function is not well understood (O'Brien and Wong, 2011). The Aβ hypothesis states that there is an imbalance between the production of Aβ and the removal of it, and that accumulation of Aβ contributes to Alzheimer's disease (Selkoe and Hardy, 2016). These Aβ oligomers can be soluble or insoluble. Although not completely understood, soluble Aβ seems to contribute more to the symptoms of Alzheimer's disease than do the insoluble Aβ proteins (Haass and Selkoe, 2007). The second hypothesis for Alzheimer's disease pathology is neurofibrillary tangles. These tangles are composed of hyperphosphorylated tau proteins. Under normal circumstances, tau proteins help with the assembly and stabilization of microtubules. However, in Alzheimer's disease when tau proteins become hyperphosphorylated, they undergo changes that cause the tau proteins to

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aggregate, leading to the formation of neurofibrillary tangles (Chong et al., 2018). Studies have also shown that the presence of Aβ can cause the hyperphosphorylation of the tau proteins. This is called the amyloid cascade, as the presence of Aβ triggers several other events that lead to neuronal death. These pathologies lead to the symptoms of Alzheimer's disease which include progressive memory loss and cognitive deficits due to neuronal death (De-Paula et al., 2012)(Figure 1).

There are little to no effective treatments for those with Alzheimer's disease. The medication that is available only provides minimal symptom relief, without treating the direct causes (Madav et al., 2019). Such medications include Donepezil, Rivastigmine, and Galantamine. The main goal of these drugs is to increase acetylcholine. Decreased amounts of acetylcholine have been noticed in the primary stages of Alzheimer's disease. Because these medications do not treat the underlying pathologies or prevent the progression of Alzheimer's disease, it is important to find a treatment that can directly target the pathologies of Alzheimer's disease.

Numerous studies are being done to try to determine the possible treatment(s) for Alzheimer's disease. The use of stem cells to create an effective treatment has increased in prevalence. The most common ways stem cells are being used are as transplants. These transplanted cells can include proliferation and replacement of dead cells, as well as include neurotrophic factors (Bali et al., 2017). This is the case with Blurton-Jones et al. work in 2009.

In one of the most important uses of stem cells, Blurton-Jones and colleagues (2009) used transgenic mice as their Alzheimer's disease model. They injected neural stem cells (NSCs) containing brain-derived neurotrophic factor (BDNF) into the hippocampus of these mice. Blurton-Jones and colleagues found that NSCs injections improved cognition, however, Aβ and tau pathologies were not affected. Instead, BDNF caused increased synaptic density in the hippocampus which helped improve cognition. Through other experiments, they found that when the gene encoding for BDNF, a neuronal growth and survival factor, was knocked down in transplanted NSCs, cognition did not improve. With this, they concluded that neural stem cells containing BDNF can improve cognition due to increased synaptic density (Blurton-Jones et al., 2009).

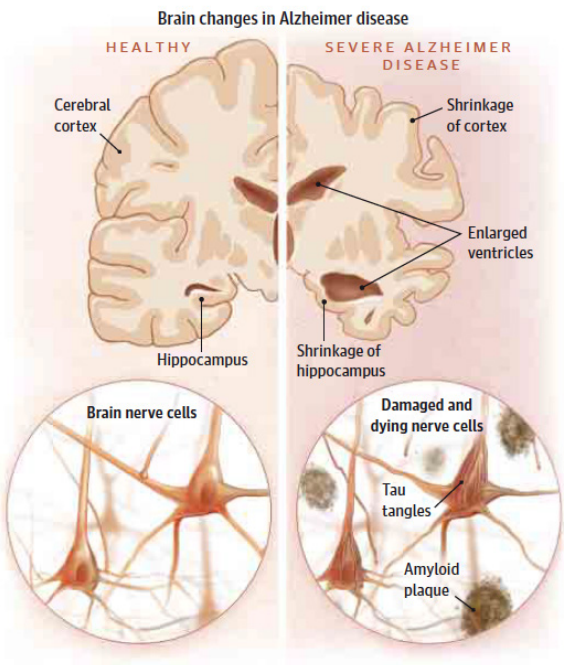

Figure 1: Amyloid plaques and tau neurofibrillary tangles contribute to the symptoms of Alzheimer's disease. Illustration of a normal brain compared to an Alzheimer's disease brain. Neuronal death is caused by the build up amyloid-β plaques and neurofibrillary tangles, which are composed of hyperphosphorylated tau proteins**.** Neuronal death is associated with enlarged ventricles and hippocampus size reduction. Together, these changes lead to the symptoms of Alzheimer's disease, predominantly memory loss and cognitive deficits. Reproduced with permission from [JAMA. 2015 313(14): 1488]. Copyright©(2015) American Medical Association. All rights reserved.

Methods

This methods section is paraphrased from Blurton-Jones et al., (2009) except where noted.

Mice

Blurton-Jones et al. used two different types of mice, wild type mice and aged triple transgenic mice (3xTg-AD). The 3xTg-AD mice had PS1, APP, and tau transgenes expressed (Oddo et al., 2003). Mutations in the PSEN1 gene, which encodes the PS1 protein, are the most common cause of familial Alzheimer's disease (Kelleher and Shen, 2017). There were four different experimental groups within this study. The control mice consisted of wild type **vehicle** injected mice and wild type NSCs injected mice. The experimental mice consisted of 3xTg-Vehicle injected mice and 3xTg-NSCs injected mice.

Vehicle: a substance, usually without therapeutic action, used as a medium to give bulk for the administration of medicines

Neural Stem Cells and Mouse Injections

Blurton-Jones et al. (2009) received the NSCs from Dr. Young. The authors used two different kinds of NSCs: unaltered NSCs and those with a knock-down of BDNF gene expression. To knock-down the BDNF, they used BDNF shRNA. shRNA is a kind of RNAi. When applied to NSCs, the BDNF shRNA knocked down the expression of the BDNF protein. To do this, empty viruses (lentiviral particles) had BDNF shRNA placed into their plasmid. 24 hours after shRNA placement, these lentiviral particles were applied to NSCs. After allowing the particles to be applied to NSCs overnight, the **transduced** NSCs were transplanted into mice. 18-month-old mice were injected stereotactically with 100,000 NSCs, BDNF shRNA NSCs, or vehicle into their hippocampi. Injections into the hippocampi of the mice were done because the hippocampus is crucial for the storage of old memories and the formation of new ones.

Transduced: process by which foreign DNA is introduced into a cell by a virus or viral vector

Behavior Assays

One month after transplantation, mice were habituated and trained on the Morris water maze and context-dependent novel object recognition, both of which are hippocampal-dependent behavioral tasks

Morris Water Maze and Probe Trial Testing

The Morris Water Maze (MWM) was designed to assess spatial learning. One reason this method was used is because there is extensive evidence that the MWM accurately measures hippocampus spatial navigation and long-term memory (Vorhees and Williams, 2006). The objective of this maze was for the mice to use their memory to find the escape platform in a pool of opaque water. Blurton-Jones et al. (2009) first had mice acclimated to a one meter circular pool. During training, the mice performed four trials a day. The mice were to find and climb onto a 12 x 12 platform that was under the water. After six days of training, the platform was removed and the mice were tested 24 hours later to assess memory. This assessment is referred to as probe trials. As was done in this paper, it is most common to give these probe trials 24 hours after the final training trial. For the probe trials, the time to reach the former platform location was recorded. The number of times the mouse crossed the former platform location was also recorded. This data was then analyzed by ANOVA and Fisher's probable least-squares difference (Fisher's PLSD) posthoc test.

Context-Dependent Novel Object Recognition

Novel object recognition tests (NOR) are used to study learning and memory. The objective

of this test is to see how much time the mice spends with an object they are not familiar with, the novel object (Antunes and Biala, 2012). Blurton-Jones et al. (2009) put mice in a round cage with two identical round balls for five minutes. The mice were then put into a square cage with two identical square cubes for another five minutes. After 24 hours, the mice were placed in either the square or the round cage with the round ball and the square cube. When a mouse is placed in the round cage, the square cube is considered novel, and vice versa for when the mouse is placed in the square cage. The time spent with the novel object and the in context object was recorded and calculated. These calculated values were then put through ANOVA and Fisher's PLSD posthoc test.

Statistical Analysis of Behavioral Assays

Analysis of variance or ANOVA is a statistical method that compares datasets. It compares two or more populations, comparing the means and the variance between these populations. In other words, ANOVA gives a P-value indicating the chance that there is a significant difference among populations. A P-value less than 0.05 indicates that there is a significant difference between populations. The Fisher PLSD posthoc test was used alongside ANOVA. While ANOVA indicates that one or more populations are significantly different than the others, it does not tell exactly which population(s) is significantly different. Fisher's PLSD posthoc test gives a P-value for all pairwise comparisons, taking into account the number of populations being compared. Again, P-values less than 0.05 are needed to indicate a significant difference.

Biochemical Assays

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA is a technique that can detect and measure substances like peptides, antibodies, and proteins. Blurton-Jones et al. (2009) used an indirect method of ELISA, specifically the sandwich method, to measure soluble and insoluble levels of Aβ40 and Aβ42. This involved coating the wells of a polystyrene plate with a specific antibody and then allowing a specific antigen to bind. Once the antigen was attached to the specific antibody, an

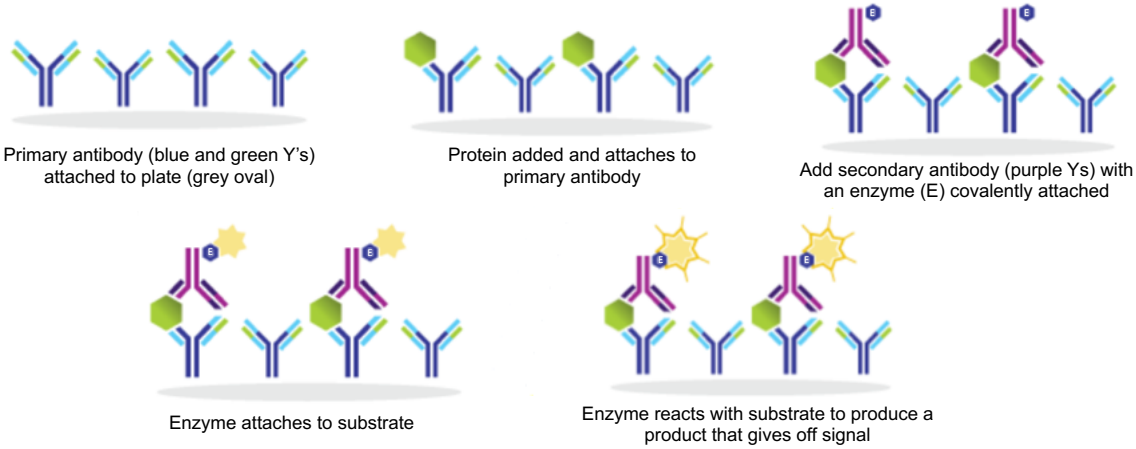

Figure 2: Sandwich ELISA can be used to detect and measure levels of proteins. Blue Y's are primary antibodies, and purple Y's are secondary antibodies. Green hexagons are protein of interest. Small indigo hexagons on secondary antibodies are enzymes. Yellow stars are substrates. Brightened yellow star represents substrate that has been converted to a reporter that gives off a signal. Adapted from https://www.raybiotech.com/files/images/How-it-works/sandwich-elisa.png.

unlabeled primary antibody was introduced and became attached to the antigen. The primary antibody was then recognized by a secondary antibody conjugated to an enzyme. Once a substrate mixture was added, a product was produced to report the presence of any antibody-antigen interactions (https://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-elisa.html). With this, the amount of soluble and insoluble Aβ40 and Aβ42 was measured. This sandwich method has greater signal amplification, and greater sensitivity of antigen detection than many other methods, because multiple secondary antibodies can bind, meaning more reporter is produced (Figure 2).

Immunofluorescence Microscopy and Labeling

Immunofluorescence microscopy is a technique used to assess the localization and expression levels of a protein. To aid in visualizing the proteins, antibodies were fluorescently labeled. A primary antibody was attached to the protein of interest. Multiple secondary antibodies containing a fluorescent tag were then attached to the primary antibody. These fluorescent tags then absorb light, which raises them into an excited, high energy-state. As they come down from the excited state, they emit photons of light. This light can be seen using an epifluorescence microscope (http://vlab.amrita.edu/?sub=3&brch=70&sim=1060&cnt=1).

Western Blot

A western blot is a technique to identify proteins. To do this, a protein mixture is applied to a gel and then proteins are separated by electrophoresis. The two most common ways proteins are separated are by size and charge. Blurton-Jones et al. (2009) used SDS-page as their gel type. SDS is a detergent that binds to proteins and covers their charge, making them all negatively charged. Thus, the separation using SDS-page gel is caused by the size of the proteins. After the proteins are separated by electrophoresis, they are transferred to a membrane. The membrane can be exposed to antibodies for the purpose of detecting target proteins. The antibodies are conjugated with fluorescent labels that emit light, thus allowing detection of the proteins using light sensitive film.

Results

Transplanted NSCs Improve Cognition

The goal of the first experiment was to determine if transplanted NSCs improved cognition in normal adult mice (wild type) and those containing Aβ plaques and neurofibrillary tangles (3xTg) (Blurton-Jones et al., 2009). Four treatment groups were used. Wild type mice injected with NSCs, wild type mice injected with vehicle, 3xTg mice injected with NSCs, and 3xTg mice injected with vehicle. Mice were given injections into their hippocampus, and after one month were trained and tested on the Morris water maze (MWM), and context-dependent novel object recognition task.

The objective of the MWM was for the mice to remember where the escape platform was in a pool of opaque water. Their ability to learn was tested during six days of training with the platform present, also known as training tests. Learning was measured by the time it took to reach the platform during training tests. Their memory was tested after 24 hours with the platform removed, also known as probe trial tests. Memory was measured by how long it took them to get to the former platform and the number of times the mice crossed the former platform location in the probe trial tests. Vehicle-injected 3xTg mice showed significant impairments in both MWM tests compared to the vehicle-injected wild type mice. However, NSC-injected 3xTg mice exhibited improved learning and memory

in both the MWM training and probe trial test compared to the vehicle-injected 3xTg mice (Figure 3-A-B). NSC-injected 3xTg mice also crossed the former platform location during probe trial testing almost as twice as often as vehicle-injected 3xTg mice (Figure 3-C). Wild type mice did not exhibit memory problems, and the injection of NSCs did not alter or improve their performance (Figure 3).

To test another aspect of behavioral memory, Blurton-Jones et al. (2009) used context-dependent object recognition. The objective of the context-dependent novel object recognition task was to see how much time the mice spent with an unfamiliar object. Mice were exposed to two identical round objects in a round cage, followed by exposure to two identical square objects in a square cage. After a period of 24 hours, mice were placed in one of the two cages with a round and a square object, and they were tested on their ability

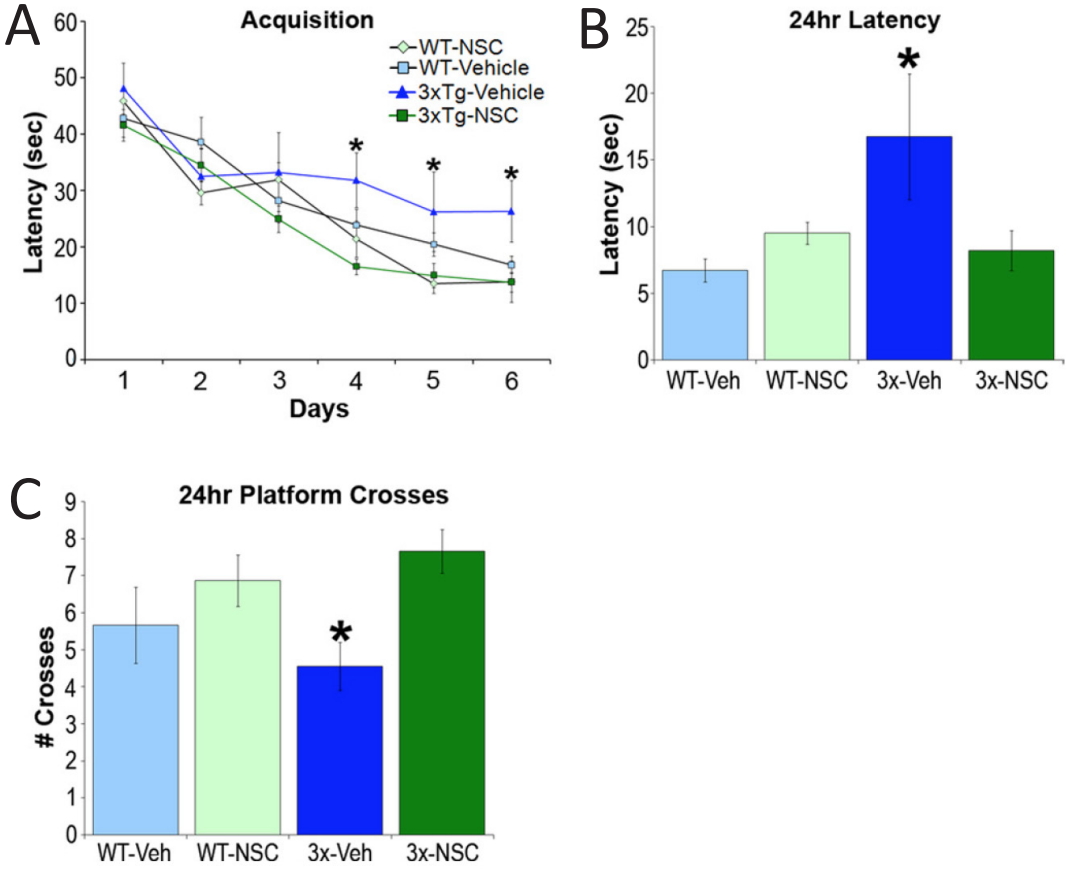

Figure 3: NSC transplantation improves cognition. Experiments using the Morris water maze. **(A)** Training period latency, or time from release of mice into the pool until platform was found. 3xTg-NSC mice exhibited significantly shorter latencies during the last days of training, compared to 3xTg-Vehicle mice (ANOVA, $P < 0.04$, FPSLD $P < 0.029$). **(B)** Results of probe trial test. After a 24 hour rest, mice were tested on how long it took them to reach the former platform location. 3xTg-NSC mice exhibited significantly shorter latencies than 3xTg-Vehicle mice, but 3xTg-NSC mice performed the same as the wild type mice (ANOVA, $P = 0.042$, FPLS $P = 0.010$) **(C)** The number of times mice crossed the former platform location. 3xTg-NSC mice crossed the former platform location significantly more times than 3xTg-Vehicle mice (ANOVA, $P = 0.014$, FPLSD $P = 0.002$). The asterisks in A, B, and C indicate a significant difference between 3xTg-Vehicle and 3xTg-NSC mice. Error bars represent a 95% confidence interval. Adapted by permission from Copyright Clearance Center: Springer Nature, Blurton-Jones et al., 2009.

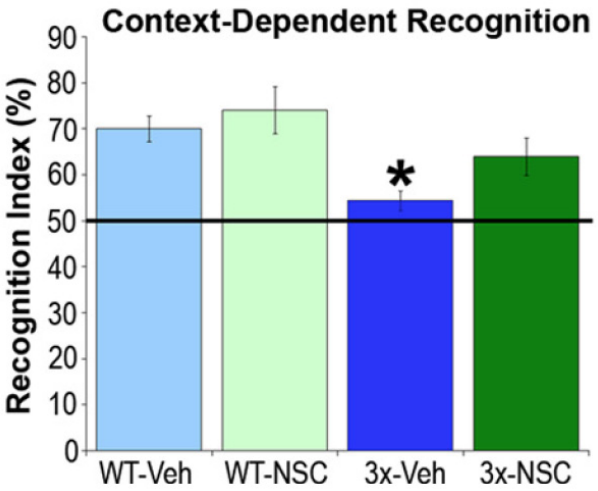

Figure 4: Context-dependent object recognition concludes that NSC transplantation improved cognition. After 24 hours, mice were placed in a round cage with a round object and square object, and time spent regarding the objects were recorded. 3xTg-Vehicle injected mice spent roughly 50% of their time exploring both objects. 3xTg-NSC injected mice spent significantly more time at the unfamiliar object over the familiar object (ANOVA, P = 0.0047, FPLSD P = 0.041). Asterisks represents a significant difference between 3xTg-Vehicle and 3xTg-NSC injected mice. Error bars represent a 95% confidence interval. Adapted by permission from Copyright Clearance Center: Springer Nature, Blurton-Jones et al., 2009.

to recognize the now out-of-context object. For instance, if mice were placed in the round cage with a round and square object, the square object is the out-of-context object. 3xTg-Vehicle injected mice spent 54% of their time studying the out-of-context object, while 3xTg-NSC injected mice exhibited significant improvement in behavioral deficits, marked by significant more time studying the out-of-context object (Figure 4). Therefore, Blurton-Jones and colleagues demonstrated that transplantation of NSC into the hippocampus of 3xTg-AD mice with Aβ plaques and neurofibrillary tangles improved cognition.

Aβ and Tau Pathologies Are Not Affected by NSC Transplantation

The goal of the next experiment was to determine if NSC transplantation had an effect on the Aβ and tau pathologies that are present in mice with Alzheimer's disease. Increased levels of hyperphosphorylated tau proteins and Aβ proteins are associated with the symptoms of Alzheimer's disease (De-Paula et al., 2012). Therefore to reduce symptoms and find a treatment, the goal is to reduce these protein levels. To test if this reduction was achieved, several different biochemical analyses were required.

Immunofluorscent microscopy was used to determine if Aβ plaque loads were effected with NSC transplantation. It revealed that Aβ plaque load did not change with NSC-injected mice compared to vehicle-injected mice (Figure 5). To determine if NSCstransplantation affected tau pathology, as well as Aβ pathology, a western blot was used. Western blots are used to identify and also quantify the expression levels of proteins. Western blots are used in combination with electrophoresis, which helps identify the charges and sizes of proteins. An SDS-page gel was used so that the proteins were identified by size, and not charge. Western blots were used to assay total human tau (hTau) and APP. Western blots revealed there was no difference in the levels of APP between the 3xTg-Vehicle injected mice and the 3xTg-NSC injected mice. There was also no difference in the levels of hTau protein between the 3xTg-Vehicle injected mice and the 3xTg-NSC-injected mice (Figure 6). With this, Blurton-Jones et al. concluded that transplanting NSCs had no effect on the Aβ and tau pathologies because the protein levels did not decrease.

Cognitive Improvement Accompanied by Increased Hippocampal Synaptic Density and Elevated BDNF Levels

The goal of the third experiment examined the synaptic density and the levels of BDNF. Since NSC transplantation did not affect Aβ or tau pathologies, Blurton-Jones et al. (2009) hypothesized that NSCs might instead be targeting synaptic connectivity. Synaptic

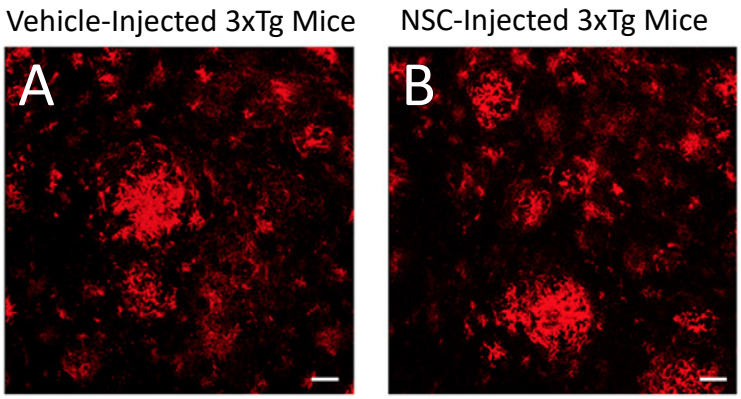

Figure 5: Aβ plaque load is not altered by NSC transplantation. Immunofluorescent microscopy revealed that there was no difference in plaque load between vehicle-injected **(A)** and NSC-injected **(B)** 3xTg mice. Note the similar levels of red fluorescence labeling the plaques in both samples. Adapted by permission from Copyright Clearance Center: Springer Nature, Blurton-Jones et al., 2009.

connectivity correlates with synaptic density in that the more connections there are in the synapses, the denser the synapses will appear. To examine synaptic density, fluorescent labeling of the presynaptic protein, synaptophysin, was used. As the name suggests synaptophysin is common in synapses, and the amount of synaptophysin correlates with synaptic density. Synaptophysin was examined by Z-stack confocal microscopy. Z-stack confocal microscopy gives spatial information and therefore a much more detailed view of protein expression, than can be viewed compared to widefield fluorescence microscopy (https://www.biotek.com/resources/application-notes/z-stacking-of-single-plane-digital-widefield-fluorescent-images/). Blurton-Jones et al. took a sliced portion of the hippocampus to use in Z-stack confocal microscopy. Imaging was followed by measuring the optical density of synaptophysin. 3xTg-NSC injected mice exhibited an increase in synaptic density compared to 3xTg-Vehicle mice, indicated by the brightness differences (Figures 7 and 8). This suggests synaptic density increased.

BDNF is a neurotrophin that helps with the expression and responsiveness to synaptic connectivity. Other studies have shown that NSCs express high levels of BDNF (Kamei et al., 2007). Therefore, Blurton-Jones et al. examined if elevated levels of BDNF present in NSC-injected mice contributed to their increased synaptic density. They used a double-label confocal microscopy and found that transplanted NSCs continue to express BDNF which is why BDNF levels are elevated in 3xTg-NSC injected mice compared to 3xTg-Vehicle injected mice (Figure 9). With this information, Blurton-Jones et al.

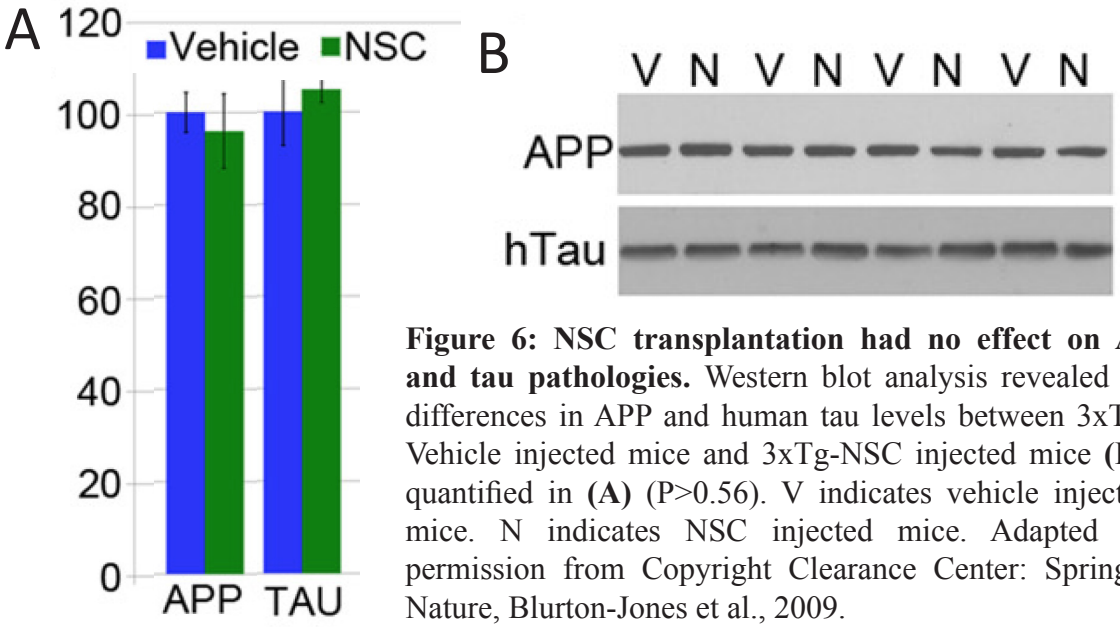

Figure 6: NSC transplantation had no effect on Aβ and tau pathologies. Western blot analysis revealed no differences in APP and human tau levels between 3xTg-Vehicle injected mice and 3xTg-NSC injected mice **(B)**, quantified in **(A)** (P>0.56). V indicates vehicle injected mice. N indicates NSC injected mice. Adapted by permission from Copyright Clearance Center: Springer Nature, Blurton-Jones et al., 2009.

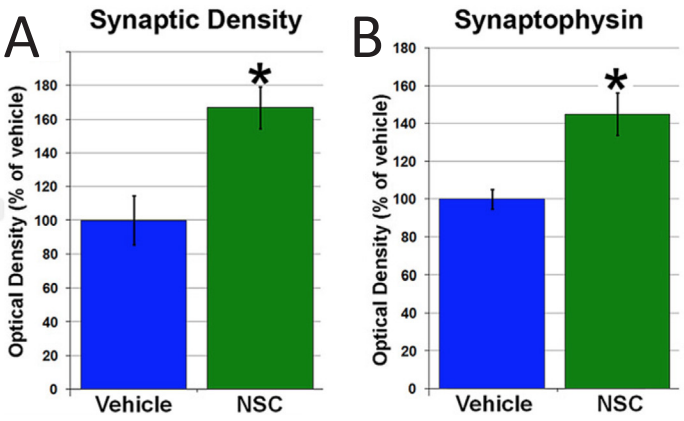

Figure 7:NSC transplantation increased synaptic density. Quantification results. **(A)** Quantification of confocal optical densitometry. 3xTg-NSC injected mice exhibited a 67% increase in synaptophysin immunoreactivity compared to 3xTg-Vehicle injected mice (P = 0.0028). **(B)** Quantification of immunoblot analysis of synaptophysin. 3xTg-NSC injected mice exhibited a 45% increase in synaptophysin compared to 3xTg-Vehicle injected mice (P = 0.01). Asterisks represent a significant difference between 3xTg-NSC and 3xTg-Vehicle injected mice. Errors bars represent a 95% confidence interval. Adapted by permission from Copyright Clearance Center: Springer Nature, Blurton-Jones et al., 2009.

concluded that NSC transplantation caused improved cognition due to increased synaptic density resulting from the elevated levels of BDNF present with the hippocampus of the brain.

BDNF Is Essential for NSC-Induced Improved Cognition

The goal of the final experiment was to determine if BDNF was required for the improved cognition, or if it was something else in the NSCs that improved cognition in mice. To do this, Blurton-Jones et al. (2009) performed a loss of function experiment where a type of interfering RNA, sh-BDNF, was used to knock-down the expression of BDNF in NSC-injected 3xTg mice. This was injected into the hippocampus of the 3xTg NSC-injected mice. After one month, mice were trained and tested using the MWM. Because Blurton-Jones et. al hypothesized that elevated BDNF was responsible for the increased synaptic density, they also decided to use Z-stack confocal microscopy and densitometry to quantify synaptophysin.

As expected 3xTg-NSC injected mice performed significantly better than 3xTg-Vehicle injected mice during the MWM training period. Interestingly, BDNF-shRNA NSC injected mice exhibited no improvement compared to 3xTg-Vehicle injected mice, and performed significantly worse than 3xTg-NSC injected mice (Figure 10-A). During probe trial testing, shBDNF-NSC injected mice performed at a level in between that of 3xTg-NSC injected mice and 3xTg-Vehicle injected mice, while 3xTg-NSC injected mice found the former platform significantly faster than 3xTg-Vehicle injected mice (Figure 10-B). Also, 3xTg-NSC injected mice crossed the former platform location significantly more times than both the shBDNF-NSC injected mice and the 3xTg-Vehicle injected mice (Figure 10-C).

Next, Blurton-Jones et al. analyzed the synaptophysin present within the synapses. As expected, 3xTg-Vehicle injected mice exhibited significantly less synaptophysin than 3xTg-NSC injected mice (Figure 8-A-B). However, shBDNF-NSC injected mice exhibited significantly less synaptophysin than 3xTg-NSC injected mice, but significantly more than 3xTg-Vehicle injected mice (Figure 8-C). Therefore, Blurton-Jones and colleagues concluded that BDNF is required to increase synaptic density.

Discussion

The goal of the Blurton-Jones et al. (2009) study was to see if NSC transplantation had an effect on cognition in an Alzheimer's mice model system. They demonstrated that

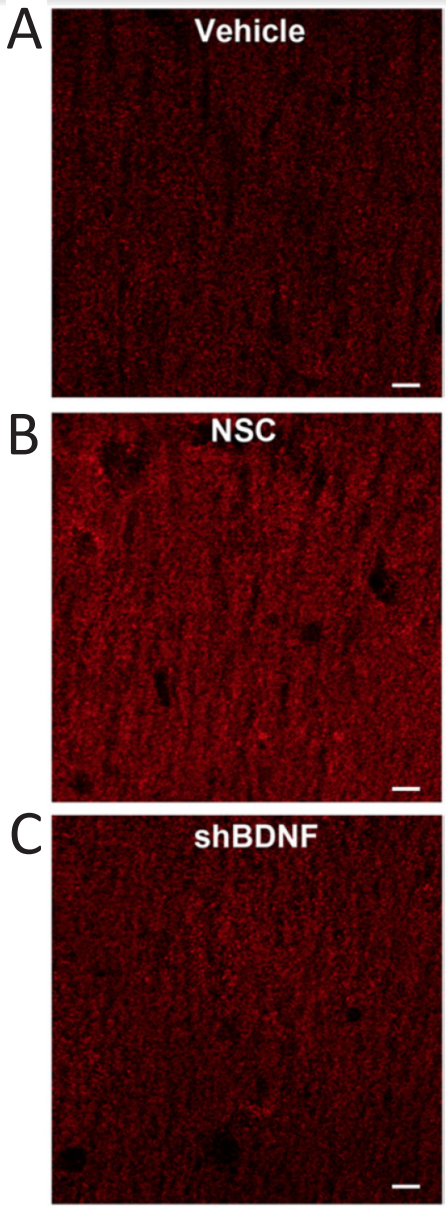

Figure 8. Injection of shBDNF reveals a intermediate level of synaptic density. Measurement of synaptophysin present within the hippocampus of 3xTg-AD mice. 3xTg-Vehicle injected mice **(A)** exhibited significantly less synaptophysin immunoreactivity than 3xTg-NSC injected mice **(B)**, ANOVA, $P < 0.0001$, FPLSD $P = 0.0001$). shBDNF 3xTg-NSC injected mice exhibited an intermediate level of synaptic density **(C)**. Expression of synaptophysin immunoreactivity was significantly greater than vehicle-injected mice ($P = 0.014$) but also significantly less than control NSC-injected mice ($P = 0.0093$). Note the brightness in NSC compared to shBDNF and vehicle. shBDNF is brighter than vehicle, but not as bright as NSC. Adapted by permission from Copyright Clearance Center: Springer Nature, Blurton-Jones et al., 2009.

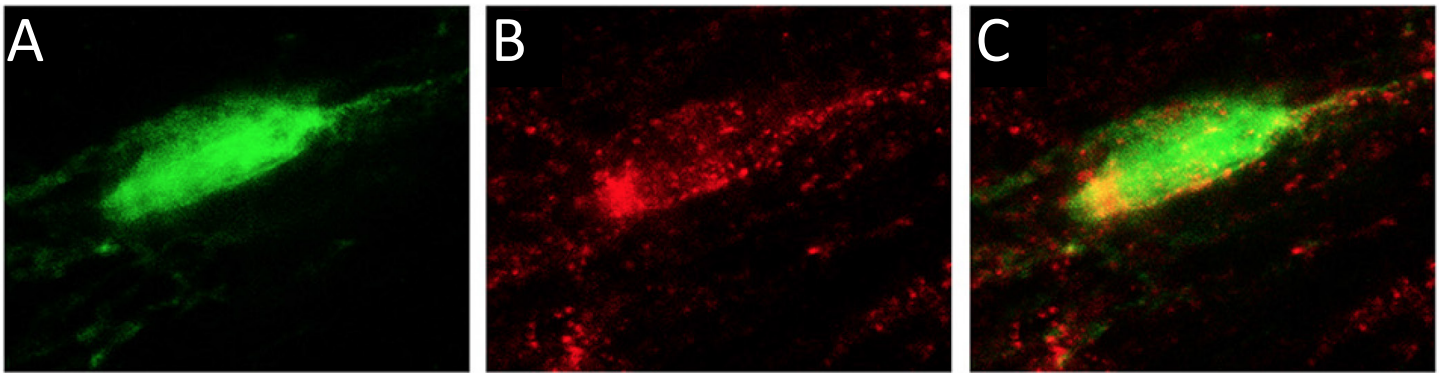

Figure 9. Neural stem cells produce BDNF. Double-label confocal microscopy was used to detect NSCs that continued to express BDNF. **(A)** Green represents NSCs expressing green fluorescence protein. **(B)** Red represents BDNF with red fluorescence antibodies. **(C)** Green and red overlapping representing NSCs and BDNF. Yellow represents NSCs that are synthesizing and secreting BDNF. Adapted by permission from Copyright Clearance Center: Springer Nature, Blurton-Jones et al., 2009.

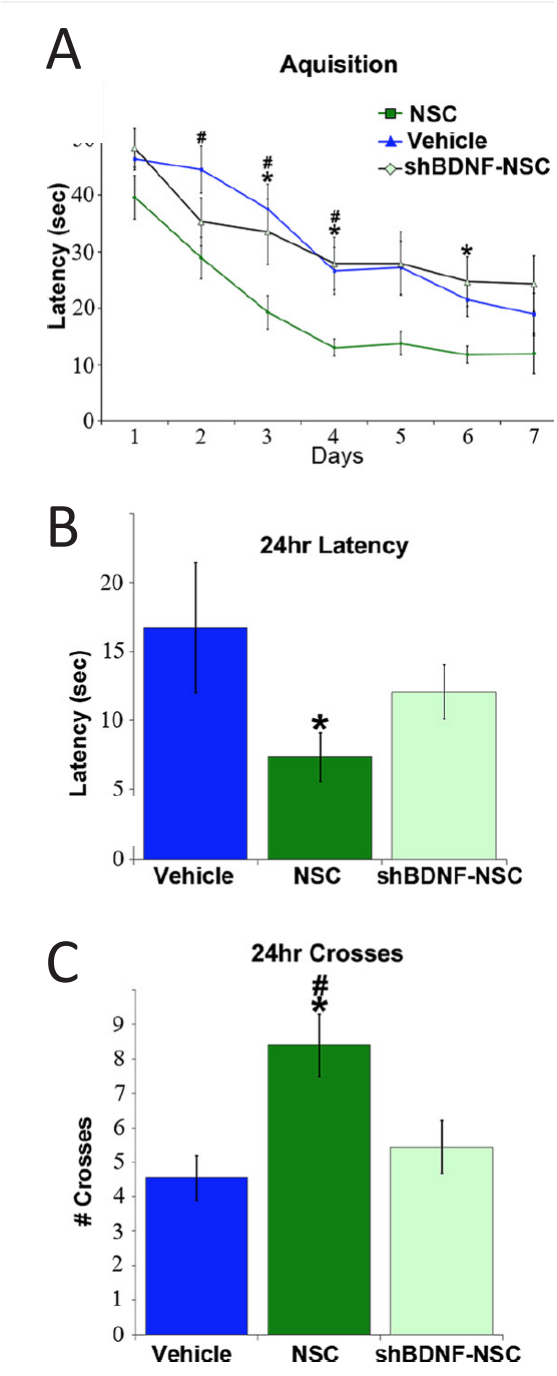

Figure 10: BDNF is necessary for NSC-induced improved cognition. Experiments using the Morris Water Maze. **(A)** Training period latency until platform was found. 3xTG-NSC injected mice exhibited shorter escape latencies on days 3-6 of training compared to 3xTg-vehicle injected mice (ANOVA $P = 0.027$, FPLSD $P < 0.03$). In contrast shBDNF NSC-injected mice showed no improvement in cognition and were impaired compared to NSC-injected mice (FPLSD $P < 0.013$). **(B)** Results of probe trial testing. 3xTg-NSC injected mice found the former platform location significantly faster than 3xTg-vehicle injected mice (FPLSD $P = 0.037$). shBDNF NSC-injected mice performed at an intermediate level that was not significantly different from 3xTg-vehicle injected mice (FPLSD $P = 0.29$). **(C)** The number of times mice crossed the former platform location. 3xTg-NSC injected mice crossed the former platform location significantly more then both 3xTg-vehicle injected and shBDNF NSC-injected mice (ANOVA $P = .0049$, FPLSD $P < 0.0136$). Asterisks represent a significant difference between NSC-injected mice and vehicle-injected mice. Number sign represents a significant difference between NSC-injected mice and shBDNF NSC-injected mice. Error bars represent a 95% confidence interval. Adapted by permission from Copyright Clearance Center: Springer Nature, Blurton-Jones et al., 2009.

NSCs improved cognition through their synthesis and secretion of BDNF. BDNF plays a vital role in the growth and survival of neurons, as well as helps with expression of synapse connectivity. Interestingly, NSC transplantation had no effect on either Aβ plaques or neurofibrillary tangles, structures that have been implicated in Alzheimer's pathology. Instead, improved cognition was caused by increased synaptic density which was mediated by the increased levels of BDNF.

We, as scientists, do not know the exact cause of Alzheimer's disease. The Aβ plaque and neurofibrillary tangle hypotheses are just those, hypotheses. It is unknown if Aβ plaques and neurofibrillary tangles are the direct causes of Alzheimer's disease and

its symptoms. A strength of these hypotheses is that Kemppainen et al. (2007) found that plaques and tangles can accumulate within the brain many years before a person is clinically diagnosed with Alzheimer's. This provides strong evidence that the plaques and tangles can possibly lead to the symptoms of Alzheimer's disease. Another strength to the Aβ hypothesis is that in a separate study, Blurton-Jones et al. found that when NSCs were genetically modified to express neprilysin, an Aβ degrading enzyme, Aβ proteins were reduced and synaptic density was increased (Blurton-Jones et al., 2014). For this reason, there is much research being done regarding these plaques and tangles. A potential weakness to these hypotheses is this study. Blurton-Jones et al. (2009) concluded that cognition improved because synaptic density increased due to the NSCs secreting BDNF. NSCs had no effect on the Aβ plaque and tau pathologies, suggesting that maybe these pathologies are not the main causes of symptoms.

As of 2009, when Blurton-Jones et al. published this paper, there was little research using stem cells directed at Alzheimer's disease. For this reason, Blurton-Jones and colleagues' work was pioneering. It was originally thought that stem cells would be used to replace dead cells (Martino and Pluchino, 2006) but Blurton-Jones et al. (2009) demonstrated that NSCs improved cognition through a bystander effect. This means that NSCs improved the Alzheimer's symptoms indirectly. The NSCs improved cognition because the BDNF targeted the synapses, indicated by the increased synaptic density. Other studies have also concluded that the beneficial effects of NSCs happen this way (Martino and Pluchino, 2006). Therefore, this study helped further our understanding of how NSCs work.

A second reason why this paper is exciting is because when this paper was published in 2009, NSCs and one other treatment were the only ones that caused improved cognition in Alzheimer's model mice. The other treatment was **immunotherapy**. In one study using immunotherapy, many different antibodies were passively or actively injected into 3xTg mice (Oddo et al., 2006). This study demonstrated that when soluble Aβ and tau were reduced, cognition improved. According to the Aβ cascade hypothesis, accumulation of Aβ proteins leads to the hyperphosphorylation of tau proteins causing the neurofibrillary tangles. However, when soluble Aβ alone was reduced, cognition did not improve. This suggests that tau pathologies also play a large role in neurodegeneration. Finding a treatment for Alzheimer's disease also means finding a treatment for other tau diseases. Other diseases include frontotemporal dementia and corticobasal degeneration (Spillantini and Goedert, 1998). These are all characterized by the hyperphosphorylated tau proteins that are also present with Alzheimer's disease.

Immunotherapy: the treatment of disease by activating or suppressing the immune system

Blurton-Jones et al. study, along with other studies, lead to some new questions. Are NSCs and immunotherapy applicable to humans even though improved cognition was seen in mice? One study in the clinical trial phase using immunotherapy was shut down because 6% of patients developed **meningoencephalitis** (Weiner and Frenkel, 2006). Scientists use mice because there are physiological similarities between mice and humans. However, using mice to study human diseases is less reliable because mice and humans have been found to respond differently to the same treatment. More often than not, a drug that works in mice and shows a potential treatment for humans, does not work in humans (Perlman, 2016).

Meningoencephalitis: inflammation of the brain and surrounding tissues, usually caused by infection

Another question to ask is does one transplantation of NSCs improve cognition, or are multiple transplantations required over the course of a patient's lifetime? A further study should be performed to test this. Perhaps Blurton-Jones et al. could have kept some mice alive for months or years after NSC transplantation to determine if one injection is

sufficient or if multiple injections are required to keep cognition improved. Blurton-Jones et al. terminated the mice shortly after NSC transplantation to examine the brain, so it is unknown if cognition improvement would have remained or if cognition would have declined after a period of time.

In a side experiment, Blurton-Jones et al. demonstrated the BDNF alone did improve cognition, thus stem cells were not needed. If BDNF alone can be used, does this take away the need for stem cells? If so, is one injection enough to improve cognition or would multiple injections over the years be required? NSCs have the ability to synthesize and secrete BDNF. However, if there are no NSCs in the first place, would BDNF continue to be synthesized? Perhaps using stem cells would make BDNF last longer, reducing the need for more injections. For this reason, perhaps NSCs are a necessary part of the equation.

It is important to find if neurotrophic factors, such as BDNF, can be effective on their own, since the long-term implications of stem cells are unknown. A popular stem cell is induced pluripotent stem cells (iPSC). These are a person's somatic cells re-engineered to become any cell within the body. Therefore, an iPSC can be re-engineered to become a neuron or a NSC that can secrete BDNF. This is important because it does not raise ethical questions compared to using embryonic stem cells. In contrast to NSCs, which come from a donor, the use of iPSCs also reduces the chance of rejection to essentially zero because they come from the person's own body. However, induced pluripotent cells have been shown to produce **teratomas**. Teratomas have been shown to form in animals being studied, resulting in no further progress to clinical trials. A reason for this is because it has been hypothesized that teratoma formation would be even greater in humans (Wu and Hochedlinger, 2011). Teratomas are usually harmless, occur on the surface of the skin, and are easy to remove surgically. However, it is unknown what their effects would be if they occurred within the brain. Therefore, much more research needs to be done in the field of stem cells in order for them to be used in human lives.

Teratoma: A type of germ cell tumor that may contain several types of body tissue

This paper demonstrates the significance of using NSCs to improve cognition despite not having an effect on the Aβ and neurofibrillary pathologies. It raises further questions regarding the current hypotheses of Alzheimer's disease and whether the Aβ and neurofibrillary pathologies contribute to the symptoms. It also raises questions regarding further expansion of experiments performed in this study, applicability to humans, and potential long-term implications of stem cell use. Overall though, Blurton-Jones et al. have carved a path in the scientific field to hopefully find a successful treatment for Alzheimer's disease using stem cells.

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