Stem cells slow cognitive decline in Alzheimer’s disease via neurotrophin action

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Alzheimer’s disease is a growing concern with no satisfactory current treatment solution. Contemporary stem cell research offers a new arena for development in this field. Transplantation of stem cells into the damaged brain brings hope of repair to damaged neurons. This appears to operate via a ‘bystander effect’ whereby neurotrophins secreted by the cells act as a neuroprotectant, rather than a cell replacement mechanism as some have postulated. Such treatments can slow or even reverse cognitive decline. Research into neural stem cell transplantation has shown reversal of cognitive decline in animal models of disease via the mechanism of brain-derived neurotrophic factor secretion. Studies using nerve growth factor secreting stem cells have showed promising results with cognitive decline reversed in animal models of the disease. A Phase 1 clinical trial also showed promising reversal of cognitive decline in human subjects using transplantation of nerve growth factor secreting fibroblasts. Mesenchymal stem cells have also shown promise, and results from human trials are awaited. Induced pluripotent stem cells have provided a successful model of human disease in vitro. Although early results from transplant studies are encouraging, a lot more research will be needed before these preliminary advances can be translated to therapies with a strong evidence base to be used in practice.

Key words: Alzheimer’s, stem cells, transplantation, BDNF, NGF, cognitive decline

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Alzheimer’s disease is the most common form of dementia. There is no cure, and current treatments are palliative and offer a symptomatic relief of a limited duration. Pathologically, the brain suffers from loss of neurons and decreased numbers of synapses, with a build-up of plaques of amyloid-beta protein and fibrillary tangles of tau protein, leading to disruption of normal function and eventually cell death. This occurs in specific areas of the brain including the hippocampus, basal forebrain cholinergic neurons and areas of the cortex that are involved in learning and memory. The disease manifests in problems of memory and learning, and general cognitive decline that worsens over time.

With the number of sufferers set to rise to 42.2 million worldwide by 2020 and 81.1 million by 2040 (Ferri et al., 2005), this clearly poses a great problem for healthcare providers, not least one of funding to support large numbers of patients who need long-term care solutions. Additionally, and arguably more importantly, Alzheimer’s puts a huge emotional burden on caregivers and family members, who are forced to watch loved ones slowly lose their abilities and personalities. With an estimated one in two people over the age of 85 set to suffer from the disease (Zhu and Sano, 2006), this predicament may be all too close to home for many of us.

Currently, NICE recommend only four treatments for Alzheimer’s. Three of the four: donezepil, rivastigmine and galantamine are acetylcholinesterase inhibitors. These prolong the presence of acetylcholine (a neurotransmitter involved in memory function) at the synapse, by blocking the enzyme acetylcholinesterase. This slows deterioration of cognitive function in patients with mild to moderate disease (Wolfson et al., 2002; Birks, 2006; Prvulovic and Schneider, 2014). The fourth, memantine is an NMDA receptor antagonist. It prevents...
excitatory glutamate neurotoxicity (Choi, 1992) and has been shown to be effective in severe dementia (Di Santo et al., 2013). With these treatments only able to slow cognitive decline for a period, before allowing the disease to resum its course, there is real need for disease modifying therapies that could halt cognitive decline or even reverse its course. A potential new drug, Dimebon, an antihistamine, recently showed promise in early trials (Doody et al., 2008), but then it failed in Phase III clinical trials (Miller, 2010). It would seem that a new approach to the problem is needed.

### Neurotrophin activity

Research has shown that despite the obvious pathological hallmarks of protein plaques and fibrillary tangles in the brain, the best physical marker of disease progress is in fact loss of synapses between neurons, which most closely correlates with cognitive decline (Terry et al., 1991; Perez-Cruz et al., 2011; Neuman et al., 2014). So a possible treatment avenue could be to increase brain synaptogenicity to try and reverse this decline. In the healthy brain, synapse formation is modulated by neurotrophins (Arancio and Chao, 2007). This idea is also supported by the so-called neurotrophic factor hypothesis of AD, which states that insufficient levels of neurotrophic factors in crucial regions such as the hippocampus result in degeneration of neurons, and also indirectly leaves them vulnerable to damage (Yuen et al., 1996; Schinder and Poo, 2000).

Examples of two neurotrophins are nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF). NGF was the first nervous system growth factor to be discovered. It is involved in the function and survival of the basal forebrain cholinergetic neurons, an area affected by Alzheimer's disease. NGF stimulates a tyrosine kinase receptor expressed by these neurons (TrkA) promoting the maintenance of synaptic contact with hippocampal and cortical neurons (Schleibs, 2011). BDNF is expressed in the entorhinal cortex and is anterogradely transported to the hippocampus where it is also involved in synapse plasticity and memory (Murer, Yan and Raisman-Vozari, 2001).

In Alzheimer’s, it has been shown that these key neurotrophins are only present at reduced levels (Schulte-Herbrüggen, Jockers-Scherubl and Hellweg, 2008; Kamei et al., 2007). This deficiency of neurotrophins leaves their normal role of modulating synapse formation unfulfilled, probably leading to reduced synaptic plasticity and resulting in the symptoms of cognitive decline (Kamei et al., 2007).

### Neurotrophins and stem cells

Stem cells are cells that have the ability to self-renew and differentiate into many different cell types. They can be divided into two broad groups—pluripotent stem cells, which have the ability to differentiate into any cell type, and multipotent stem cells, which can differentiate into a limited range of cell types, depending on their germal origin (Martinez-Morales et al., 2013). For example, neural stem cells (NSCs) can differentiate into neurons, astrocytes or oligodendrocytes (Taupin, 2006).

In adults, NSCs are found in the subgranular zone and subventricular zone of the hippocampus (Taupin, 2006), part of the brain which is important for learning and memory (Deng, Aimone and Gage, 2010).

NSCs express high levels of neurotrophins including BDNF and NGF (Lu et al., 2003; Kamei et al., 2007). Transplantation of these cells into areas of pathology in the brain would therefore present a mechanism of delivery of these factors directly to the brain which avoids the difficulty of the factors being unable to cross the blood–brain barrier, a problem intrinsic to more peripheral methods of delivery. A stem cell graft would produce the neurotrophins in situ. This would theoretically help to improve synaptogenicity in the damaged areas and therefore hopefully improve the cognitive decline experienced by the patient (Chen and Blurton-Jones, 2012).

However, transplanting stem cells does pose some problems. Implanting cells that grow and divide can have the effect of producing a tumour if the growth becomes unregulated. One study compared implantation of pluripotent embryonic stem cells and embryonic stem cell-derived NSCs into the cortex of an AD mouse model. While the NSCs reduced memory deficits, the pluripotent embryonic stem cells caused worsening of the cognitive defects via teratoma formation (Wang et al., 2006). However, this does demonstrate the relative safety of using NSCs, since they are only multipotent and thus less prone to tumour formation in situ. A disadvantage of NSCs is that they cannot easily be obtained from the human brain, and so studies often use foetal NSCs, which pose ethical problems (Liras, 2010).

Another option that has been explored is to genetically modify a fibroblast cell to produce neurotrophins and then implant these modified cells into the brain as a source of neurotrophins. This technique has several advantages. Fibroblasts can easily be obtained from an adult by skin biopsy. This means that there are no associated ethical issues, and that the cells are already matched to the patient. Nevertheless, the cells do have to be genetically modified to express the correct neurotrophin, adding another stage to an already complex procedure.

Additionally, transplantation of astrocytes has also been considered as a potential avenue for treatment of Parkinson’s disease (PD). Astrocytes expressing glial-derived neurotrophic factor (GDNF) were delivered to the striatum of rat and mouse models of PD. Motor improvement was shown in the animals receiving the astrocytes, and importantly, the GDNF was not transported to other parts of the brain, as it had been with other delivery mechanisms, which could cause potential side effects (Drinkut et al., 2012). While this shows proof of concept of astrocyte transplantation as a delivery vehicle for GDNF, the authors caution that the results do not show clinical efficacy, as the treatment occurred before brain...
lesions in the animals. Further studies will be needed to evaluate whether this claim can be made.

**Neural stem cell transplantation and BDNF in the 3xTg-AD mouse model**

Blurton-Jones *et al.* (2009) investigated neural stem cell transplantation as a method to reduce cognitive decline in Alzheimer's via the action of BDNF. They used a transgenic mouse model known as 3xTg-AD to model the effects of Alzheimer's disease on the brain. These mice have many of the important pathological features of the disease including amyloid-beta plaques and neurofibrillary tangles, and experience cognitive decline. Although there is no mouse model that exactly mimics all of the features of the human disease and so a mouse will not be directly generalizable to human Alzheimer's sufferers, this model includes many important parallels and so conclusions can be drawn bearing this in mind.

The neural stem cells were grown *in vitro* and transplanted into the mice via stereotactic injection to the brain. Four groups of mice were compared—transgenic and wild-type mice were injected with the NSCs, and controls of age-matched transgenic and wild-type mice were injected with a similar volume of vehicle alone. This was an important control as it has been suggested that brain injection itself could stimulate production of neurotrophins (Cafferty, McGee and Strittmatter, 2008), which would be a confounding variable. The mice were tested on tasks designed to assess cognition including the Morris Water Maze, and context-dependent novel object recognition. They found that the vehicle-injected transgenic mice experienced significantly impaired cognition compared with their wild-type controls, showing the cognitive decline caused by the brain pathology they had been engineered to develop. The NSC transplant transgenic mice had significant improvements in cognition compared with the vehicle-injected transgenic mice, showing the benefits that the NSC transplant had brought.

The fate of the NSCs was studied in detail 5 weeks after the transplant procedure. Post-mortem tissues were studied microscopically, enabled by fluorescent markers attached to the cells. It was found that the stem cells differentiated into all types of neural cell (astrocytes, oligodendrocytes and neurons) but that the large majority differentiated into astrocytes. The stem cells did not alter the pathology of either plaques or tangles, thought by many to be the causative features of the disease, but appeared to work by affecting synaptic density in the hippocampus. Post-mortem analysis showed an increase of 67% in hippocampal synaptic density in the NSC transplanted transgenic mice compared with their vehicle-injected controls. Synaptic density was quantified by measuring levels of synaptophysin, a presynaptic protein, in the stratum radiatum of the CA1 region of the hippocampus. This is a well-established method, previously used in major studies of Alzheimer's in both mice and humans (Terry *et al.*, 1991; Mucke *et al.*, 2000). This increase in synaptic density is an interesting discovery as all previously tested therapies have improved cognition by improving plaque or tangle pathologies in some way, and this approach does neither. This research therefore opens a new avenue of an alternative approach to therapy.

The concept of the mechanism of action of the neural stem cells via BDNF was also thoroughly tested. A microfluidic cell culture device (which facilitates axonal analysis) was used to examine axon growth *in vitro*. Neurons were exposed to three different media—a control conditioned media, NSC-conditioned media or NSC-conditioned media where immunoprecipitation had been used to remove BDNF. Axonal outgrowth quantification showed that the NSC-conditioned media highly increased axon growth and density compared with both controls. This suggests that the growth effects provided by NSCs are mediated via BDNF.

To test the same principle *in vivo*, lentiviral delivery of shRNA was used to stably produce knockdown NSCs, which produce a markedly reduced level of BDNF. Mice were injected with these stem cells, normal NSCs or an equivalent volume of vehicle. The knockdown injected mice performed significantly worse on the cognitive tasks than those injected with normal NSCs and no differently from the vehicle-injected controls. Hippocampal synaptic density was also significantly higher in the normal NSC injected mice than either of the other groups (Blurton-Jones *et al.*, 2009). This thorough testing of mechanism effectively substantiates the paper’s claim that the effects seen in improved cognition are in fact mediated via BDNF produced by the NSCs, rather than via another pathway.

This study had the advantage that the NSCs grown were tested thoroughly pre-implantation for well-established markers of multipotency sox-2 and nestin, and coexpression of GFP (green fluorescent protein), the fluorescent marker used. This shows that the cell lines grown were in fact as the researchers intended and that they could be identified accurately via fluorescent microscopy. Also the therapy was tested on mice that had extensive brain pathology of plaques and tangles. In humans, these build up for many years before manifestation of symptoms, so it is important to have a therapy that has been tested in and works despite, extensive pathology.

**Neural stem cell transplantation in other mouse models**

Work has also been done in other mouse models that try to recapitulate some of the salient features of Alzheimer's. Hampton *et al.* (2010) used a mouse model of human tauopathy P301S. This mouse has an age-related build-up of hyperphosphorylated human tau in the brain. This causes loss of neurons similar to that found in Alzheimer’s.

They found that after transplanting NSCs into the transgenic mice, there was a significant increase in number of cortical neurons compared with the non-transplanted
contralateral hemisphere of the mouse. The NSCs were able to counteract the toxic effects on the cells of the misfolded tau proteins, allowing cell survival. This is known as a neuroprotective effect. On analysis of the brains of the mice, it was found that the majority of the NSCs had differentiated into astrocytes. There was also an increase in neurotrophins, particularly GDNF (Hampton et al., 2010). As the NSCs had increased neuron number overall, but not differentiated into neurons themselves, a cell replacement mechanism of action seems unlikely. Rather, a neuroprotective effect from the astrocytes produced by the NSCs appeared to be in play, mediated by the effect of secreted neurotrophins. This is known as a bystander effect. However, there was no further study of whether GDNF itself was causing the increase in cortical neurons, so cause and effect cannot be assumed.

**Nerve growth factor and clinical trials**

NGF has also been extensively studied as a neurotrophin that could help to regenerate the damaged brain. Specifically, it has an effect on survival of basal forebrain cholinergic neurons. As this is an area affected in Alzheimer’s, it has tremendous therapeutic potential for this disease (Tuszynski, 2007).

Initial research was done in animal models. Chen et al. modified rat fibroblasts obtained by skin biopsy to secrete NGF using a murine retroviral vector containing human beta-NGF cDNA. The NGF secreting fibroblasts were then implanted into the brains of ageing rats. They found that there was an increase in the number of neurons, and that memory impairment was reduced, compared with rats receiving a graft of non-engineered fibroblasts. This showed that the secreted NGF was acting as a protectant for the neurons, increasing survival (Chen et al., 2005).

Studies have also been done in primates that are more closely related to humans, and so results are more likely to be generalizable to the human population. Tuszynski et al. lesioned the fornix of the brains of rhesus monkeys and then transplanted NGF secreting fibroblasts. In the control group, where fibroblasts that were not modified to secrete NGF were transplanted, only 25% of cholinergic neurons survived, compared with a neuron survival level of 92% in the test group. This reiterated the neuroprotective effect of NGF.

Because of the level of promising research in this area, it was felt that a clinical trial was warranted to test the therapy in humans. Tuszynski et al. (2005) conducted a Phase 1 clinical trial to test stem cell delivery of NGF as a possible therapy for Alzheimer’s. Subjects with early-stage, probable Alzheimer’s were recruited. Skin biopsies were taken from the subjects, and fibroblasts were modified to secrete NGF via retroviral vectors. These were stereotaxically injected into the cholinergic basal forebrain in one procedure. Cognitive outcomes were measured using MMSE and ADAS-Cog tests (commonly used clinical tests to assess cognitive function), and PET scans assessed activity in affected brain regions.

No adverse effects related to the NGF were reported. Two subjects suffered haemorrhage due to movement during the procedure, which was conducted while they were awake and sedated. Other procedures were performed under general anaesthesia and were completed safely.

Results showed that cognitive decline appeared to be reduced by 36–51% as measured by typical clinical tests over a mean period of 2 years, compared with preoperative cognitive decline rates. This shows the neuroprotective action of NGF in damaged areas of the brain. PET scans showed uptake of glucose was increased in areas of cell delivery. This usually decreases in Alzheimer’s (Potkin et al., 2001) as damaged areas are less metabolically active as they are not functioning correctly. On post-mortem analysis of a trial member who died 5 weeks post-surgery following a pulmonary embolism, it was found that axon sprouting occurred at site of cell delivery. This concurred with results reported from animal models. This shows that the action of the NGF produced by the cells was to encourage neuronal growth (Tuszynski et al., 2005).

However, this study does have limitations that need to be taken into account when drawing conclusions from such data. As a small, non-placebo controlled, non-blinded study involving analysis of seven subjects, bias is likely to be present in many areas.

The participants were current patients at the clinic with no random selection to avoid bias in the sample, and so are unlikely to be representative of the general population. The sample was also very small. This limits generalizability of the study. Ideally, the study should be placebo controlled with some patients receiving an injection of an equivalent volume of vehicle to act as the control group. This would give an important comparison. However, the choice of patients suffering from early stages of the disease was an important one. These patients are the ones likely to be receiving and benefiting most from the therapy if it is proved effective and so are the most important group to test it in.

Experimental conditions were different between patients, with some receiving bilateral injections of cells and some injections to the right brain only. This limits the conclusions that can be drawn from comparison of the subjects. When comparing PET scans, only some subjects received a preoperative scan to act as a control. In the subjects without preoperative scans, more limited conclusions can be drawn without the control comparison. Additionally, no data were collected for preoperative rate of decline on the ADAS-Cog test. Instead, published averages were used for comparison. This is not ideal, as there will be inter-patient variation, and published averages are unlikely to be applicable specifically to the individual patient. Therefore, more weight should be given to the MMSE results, which were collected both pre- and post-surgery, and therefore have a valid control.
However, the authors took the limitations of their study into account, drawing only tentative conclusions and recommending the need for more depth of research into the therapy.

Overall, further and larger clinical trials are clearly needed, but this therapy potentially presents an impressive advance over current treatments that improve cognition by around 5% (Mayeux and Sano, 1999) and do not act for an extended time period. In contrast, the possibility of an improvement of up to 51% sustained over at least 2 years is very encouraging.

### Recent developments with MSCs

More recently, human mesenchymal stem cells (MSCs) have been shown to release protective factors, including BDNF (Chen and Chopp, 2006), into damaged tissues including the brain (Chamberlain et al., 2007; Ylostalo et al., 2012). This makes them an ideal candidate for transplantation. They also have the benefit of being able to be obtained peripherally, from blood, adipose tissue or bone marrow (Martinez-Gonzalez et al., 2006). This means that they are easy to obtain and can be taken from the patient themselves. Moreover, they have none of the ethical issues associated with embryonic stem cells.

Animal studies have shown promising results. Lee et al. used a double transgenic mouse model of amyloid precursor protein and presenilin-1, and transplanted human umbilical cord derived stem cells into the hippocampus. They found that compared with controls injected with buffer solution, the transplant mice had significantly improved spatial learning and memory decline, as tested by the Morris water maze, escape latency, and crossing platform test (Lee et al., 2012). Interestingly, they attributed this to reversal of disease associated microglial inflammation. They found reduced levels of pro-inflammatory cytokines, increased levels of anti-inflammatory cytokines and higher numbers of alternatively activated microglia, thought to be neuroprotective (Lee et al., 2012). This provides an alternate mechanism of action to the release of neurotrophins.

This has led to two human trials of intracerebral infusion of MSCs in patients with Alzheimer’s which are currently ongoing (NEUROSTEM-AD). It will be very interesting to see the outcome of these trials.

### Other applications of stem cells

Induced pluripotent stem cells can be made by reprogramming somatic cells with forced expression of particular transcription factors to induce them into a pluripotent state. First achieved in mice (Takahashi and Yamanaka, 2006), this technique has also been applied successfully to human cells (Takahashi et al., 2007; Park et al., 2008). The technique has the advantage of being free from ethical issues surrounding embryonic stem cells, and also that the cells are already specific to the individual patient.

These cell lines can be used to provide reasonably accurate in vitro models of Alzheimer’s disease, which are crucial for further research into disease mechanism and can also be used to test potential new drugs.

A small-scale study by Goldstein’s research group took fibroblasts from two patients with familial Alzheimer’s disease, two patients with sporadic Alzheimer’s disease and two subjects without dementia. The fibroblasts were transformed into induced pluripotent stem cells (iPSCs) and these were grown to cultures of neurons. The results showed that both of the familial Alzheimer’s disease cases and one of the sporadic cases had increased levels of amyloid-beta, phosphorylated tau and glycogen synthase kinase 3-beta (GSK3-beta—the enzyme involved in hyperphosphorylation of tau) compared with the control cases (Israel et al., 2012). This shows that iPSCs can be used to successfully create an in vitro model that successfully provides the main hallmarks of the disease. We still do not fully know the cellular processes that contribute to the deterioration of the brain in Alzheimer’s disease, and models such as these provide a fantastic research opportunity to study these processes in detail.

### Conclusion

Overall, stem cell transplantation looks to be a field for future development. The ability of stem cells to secrete neuroprotective factors allows them to stimulate survival of neurons and increase synapse formation, allowing improvement of cognitive decline in the otherwise devastating condition of Alzheimer’s. MSCs may also be able to provide a neuroprotective effects, and the results from human trials are awaited. Additionally, iPSCs provide a much needed in vitro model of human disease, which can be used to study disease processes more closely, and also play a useful role in the testing of potential new drugs. It is clear that more research is needed to bring these experimental therapies into practical evidence-based treatments, but the future seems bright in this field.

### Author’s biography

R.M. has a First Class BSc (Hons) in Neuroscience and Mental Health. She is currently studying medicine at Imperial College London. Her interests include neurology and paediatrics. She is looking to become a clinician with a background in research.

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