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# Cochlear nucleus whole mount explants promote the differentiation of neuronal stem cells from the cochlear nucleus in co-culture experiments

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ABSTRACT

The cochlear nucleus is the first brainstem nucleus to receive sensory input from the cochlea. Depriving this nucleus of auditory input leads to cellular and molecular disorganization which may potentially be counteracted by the activation or application of stem cells. Neuronal stem cells (NSCs) have recently been identified in the neonatal cochlear nucleus and a persistent neurogenic niche was demonstrated in this brainstem nucleus until adulthood. The present work investigates whether the neurogenic environment of the cochlear nucleus can promote the survival of engrafted NSCs and whether cochlear nucleus-derived NSCs can differentiate into neurons and glia in brain tissue. Therefore, cochlear nucleus whole-mount explants were co-cultured with NSCs extracted from either the cochlear nucleus or the hippocampus and compared to a second environment using whole-mount explants from the hippocampus. Factors that are known to induce neuronal differentiation were also investigated in these NSC-explant experiments. NSCs derived from the cochlear nucleus engrafted in the brain tissue and differentiated into all cells of the neuronal lineage. Hippocampal NSCs also immigrated in cochlear nucleus explants and differentiated into neurons, astrocytes and oligodendrocytes. Laminin expression was up-regulated in the cochlear nucleus whole-mounts and

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regulated the in vitro differentiation of NSCs from the cochlear nucleus. These experiments confirm a neurogenic environment in the cochlear nucleus and the capacity of cochlear nucleus-derived NSCs to differentiate into neurons and glia. Consequently, the presented results provide a first step for the possible application of stem cells to repair the disorganization of the cochlear nucleus, which occurs after hearing loss.



## 1. Introduction

Sensorineural hearing loss is the most frequent sensory disorder in humans affecting nearly 10% of the European population (Roth et al., 2011). Current therapeutic strategies comprise hearing aids but no causal treatment is available (Petit, 2006). In patients suffering from this form of hearing deficit, cochlear hair cells are lost, followed by degeneration of spiral ganglion neurons. This leads to impairment in both the transduction process in the inner ear and the transmission of auditory signals to the brain. Consequently, morphological changes occur in the auditory brainstem, especially in the cochlear nucleus (Illing, 2001; Moore et al., 1994). The cochlear nucleus is located on the lateral side of the mammalian brainstem representing the first relay station for acoustic-derived neuronal input from the inner ear. Spiral ganglion neurons synapse with auditory neurons in the cochlear nucleus to relay auditory information to higher levels of the brain.

Various experiments have been performed in the field of hearing research using stem cell technologies. Endogenous inner ear stem cells were investigated (Ronaghi et al., 2012) or transplantation studies of exogenous stem cells, for example in the auditory nerve, performed (Shi and Edge, 2013). In some experiments, these newly generated or transplanted stem cells were able to restore the gap of the degenerated cells. Sometimes even functional effects were achieved (Chen et al., 2012). Furthermore it is known that the usage of stem cells can improve function in neuronal diseases, such as Huntington's Disease (Kim et al., 2008), or after spinal cord injury (Kim et al., 2007). This underlines the possibility of using stem cell technologies as a strategy to reorganize and repair the morphological changes that occur in the cochlear nucleus after hearing loss occurs.

Several transplantation and co-culture studies of exogenous stem cells have been performed in the auditory brainstem displaying good integration of the cells (Glavaski-Joksimovic et al., 2008; Jiao et al., 2011; Kaiser et al., 2014; Novozhilova et al., 2013). However since the cochlear nucleus is located near the fourth ventricle, neurosurgical approaches are difficult and only performed in the rare case of vestibular schwannoma surgery and/or auditory brainstem implantation (Matthies et al., 2013). Therefore the application of exogenous stem cells to the cochlear nucleus as therapy of hearing loss would be invasive and not practical for most patients with hearing loss. However, the use of endogenous stem cells derived from the cochlear nucleus itself is an interesting treatment strategy for restoration of morphological alterations after hearing loss.

Endogenous neuronal stem cells (NSCs) have been discovered in the postnatal cochlear nucleus of rats. These cells display all features of NSCs including the capacity to proliferate as well as to differentiate into neuronal progenitor cells and all cell types of the neuronal lineage (Rak et al., 2011). The neurogenic potential of cochlear nucleus-derived NSCs persists until adulthood as demonstrated by their ongoing capacity for neurosphere formation, BrdU incorporation, and expression of NSC-specific markers and genes (Rak et al., 2013). Cells with similar characteristics have recently been discovered in the cochlear nucleus of the mouse. These cells showed dependency in their neurogenic potential by the afferent input and were regulated by Wnt, Notch and Tgfß signalling (Ooka et al., 2012; Volkenstein et al., 2013). Thus, activation of endogenous NSCs in the cochlear nucleus is a possible treatment strategy for hearing loss.

In order to develop a stem cell based therapy, the presence of a neurogenic environment is needed to promote the differentiation of NSCs (Cao et al., 2002). Brain derived neurotrophic factor (BDNF) and Neurotrophin-3 (NT-3) are two trophic factors that are known to influence differentiation of NSCs and are expressed in the cochlear nucleus (Hafidi, 1999). Interestingly, increased levels of these trophic factors are found after ablation of the acoustic nerve (Suneja et al., 2005). In addition, fibroblast growth factor 2 (FGF2) has been demonstrated to modulate the proliferation of cochlear nucleus-derived NSCs (Rak et al., 2011) and its expression can also be regulated by stimulation of the auditory nerve (Riedel et al., 1995; Smith et al., 2002). Similar to what has been demonstrated for transplanted neuronal precursor cells in the auditory brainstem (Novozhilova et al., 2013), endogenous NSCs in the cochlear nucleus should have the capacity to differentiate into different types of neurons and glia which could restore the cellular gaps that arise in this brainstem nucleus after hearing loss occurs.

Therefore, this paper examined the neurogenic environment of the cochlear nucleus and the differentiation potential induced by brain tissue of its NSCs into various cells types of neuronal origin. Due to the fact, that endogenous stem cells are hard to trace an in-vitro co-culture system was applied to assess these questions. For this purpose cochlear nucleus-derived NSCs were co-cultured with organotypic whole-mount explants from the cochlear nucleus (Rak et al., 2011) or from the hippocampus (Stoppini et al., 1991). In addition NSCs derived from the hippocampus (Gage et al., 1995b) were co-cultured with cochlear nucleus explants. Furthermore soluble factors, which may induce differentiation, were investigated in these NSC-explant culture experiments and re-evaluated in cell culture experiments.

The present study demonstrates that NSCs derived from the cochlear nucleus can survive and differentiate after engraftment into brain tissue. Furthermore, we found that the cochlear nucleus comprises a neurogenic environment for NSCs with laminin as one of the main components expressed in the cochlear nucleus that induces differentiation of cochlear nucleus-derived NSCs. These