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Challenges for stem cells to functionally repair the damaged auditory nerve

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Abstract

Introduction—In the auditory system, a specialised subset of sensory neurons are responsible for correctly relaying precise pitch and temporal cues to the brain. In individuals with severe-to-profound sensorineural hearing impairment these sensory auditory neurons can be directly stimulated by a cochlear implant, which restores sound input to the brainstem after the loss of hair cells. This neural prosthesis therefore depends on a residual population of functional neurons in order to function effectively.

Areas covered—In severe cases of sensorineural hearing loss where the numbers of auditory neurons are significantly depleted, the benefits derived from a cochlear implant may be minimal. One way in which to restore function to the auditory nerve is to replace these lost neurons using differentiated stem cells, thus re-establishing the neural circuit required for cochlear implant function. Such a therapy relies on producing an appropriate population of electrophysiologically functional neurons from stem cells, and on these cells integrating and reconnecting in an appropriate manner in the deaf cochlea.

Expert opinion—Here we review progress in the field to date, including some of the key functional features that stem cell-derived neurons would need to possess and how these might be enhanced using electrical stimulation from a cochlear implant.

1. Introduction

Sensorineural hearing loss (SNHL) is irreversible, and is predicted to affect as many as 278 million people worldwide according to 2005 World Health Organisation estimates. This estimate is expected to increase every year with the planet's rising population and higher life expectancies. Sensorineural hearing loss can occur from a variety of factors including prolonged exposure to loud noise, antibiotic treatment with aminoglycoside drugs, or simply as a result of ageing. Critically, these factors can cause the permanent loss of cochlear hair cells, thereby breaking the normal pathway in the transmission of sound information to the brain. In addition, the hair cells are connected to the primary auditory neurons (ANs; which comprise the auditory nerve), and loss of these hair cells initiates the secondary and progressive degeneration of ANs [1]. This is important because the chief clinical treatment for SNHL is a cochlear implant, which directly stimulates the ANs in the absence of hair cells. Accordingly, the health and integrity of ANs is considered to be one of the factors affecting cochlear implant performance [2], and thus, preserving a population of robust neurons is an important factor in improving outcomes with this neural prosthesis.

Although the cochlear implant is capable of electrically stimulating surviving ANs following hair cell loss, the auditory nerve progressively degenerates resulting in small numbers of surviving ANs in long-term deafened animals [3,4]. Several studies have demonstrated that the infusion of neurotrophic factors into the cochlea can rescue primary ANs from

degeneration [5–9], however, this survival effect is lost following the cessation of treatment [10] and longer-term delivery strategies are currently being investigated [11,12]. Whilst the rate of AN degeneration is much slower in humans [13,14], in circumstances where there is a lengthy delay between hearing loss and clinical intervention, and/or a severe degree of AN degeneration, there is a reduced window for rescuing ANs with neurotrophins. Collectively, these data support the investigation of cell replacement therapies for AN degeneration and/or loss after severe-to-profound SNHL. It is worthwhile noting that stem cells may potentially be applied to replace damaged/degenerated hair cells in the deaf mammalian cochlea, with a view to regenerating a fully-functional cochlea (without the need for a cochlear implant). Whilst outside the scope of this review, such a therapy is being actively investigated by others, with promising results [15–17].

Replacement of ANs using stem cells may also prove useful for the more recently discovered auditory nerve disorder, auditory neuropathy [18,19]. Hearing impairment resulting from auditory neuropathy affects a small percentage of individuals who suffer from severe-to-profound SNHL (~8% according to a recent review [20]), which is characterised by normal outer hair cell function but perturbed auditory nerve function [18]. Patients diagnosed with auditory neuropathy routinely have poor speech recognition [18,21]. Whilst the precise mechanism(s) underlying the cause of auditory neuropathy are still not yet fully understood, the disorder is hypothesised to involve defects at the level of the inner hair cells, the inner hair cell-AN fibre synapses, the ANs, the AN fibres or any combination of these factors [18]. In cases where the inner hair cells and ANs are severely damaged, stem cells may provide replacement neurons for stimulation with a cochlear implant, thereby facilitating auditory input into the brain.

The use of stem cells to replace lost or degenerating ANs is not straightforward, and includes overcoming several major challenges including differentiation of cells into an appropriate neural phenotype (including specific electrophysiological characteristics), successful delivery into the deaf cochlea, and functional integration of these new neurons with the correct endogenous structures in the brain (including avoiding immunorejection). Whilst there have been several *in vitro* reports of stem cell differentiation toward an auditory neural lineage [22–25] and synapse formation on appropriate tissues [22,26,27], further *in vivo* studies are required to thoroughly demonstrate that the above-mentioned challenges can be overcome. For clarity, the basic anatomical features of the normal hearing mammalian cochlea are illustrated in Fig. 1.

2. Replacement of ANs using stem cells

There is a window of opportunity after hair cell injury or damage to rescue the ANs from degeneration, thus preserving the maximum numbers possible for electrical stimulation via a cochlear implant. Such minimally invasive therapy could prove useful if long-term delivery strategies are identified and applied with a cochlear implant [12,28]. However, in cases where the ANs are severely depleted, stem cells may provide a source of replacement neurons to the deaf cochlea which can be electrically stimulated with a cochlear implant [29]. This idea is not new, and many laboratories have now published studies describing the delivery of exogenous stem cells into the mammalian cochlea for the replacement of degenerating ANs. These studies, including the delivery strategies used and the study outcomes, are summarised in Table 1. Despite differences in the type of stem cell delivered and the transplantation technique adopted, the collective results from these studies support several main conclusions: 1) several exogenous stem cell types can survive in the deafened mammalian cochlea, 2) stem cells are capable of extensive migration/dispersal following their delivery into the cochlea, 3) a proportion of stem cells express neuronal and glial

proteins following their transplantation *in vivo*, and 4) transplantation of stem cells into the cochlea elicits a small, localised tissue response (if at all).

Initial attempts to transplant exogenous cells into the cochlea adopted a conservative surgical approach, infusing cells directly through the round window membrane into the scala tympani, in an attempt to minimise trauma to the delicate cochlear structures. Whilst cell survival was reported, the delivery of cells into the scala tympani frequently resulted in the extensive dispersal of cells within the cochlea, and/or low detection of transplanted cells in the target site [30–32]. More recent attempts delivering stem cells into the cochlea have investigated a more invasive approach into the modiolus, auditory nerve, or Rosenthal's canal ([31,33–36]; Fig. 1). While successful in terms of improving the numbers of exogenous cells detected within the target site, these studies also report the extensive dispersal of cells following transplantation. Several of these studies also report the presence of a localised tissue reaction, presumably due to the additional trauma necessary to deliver cells through the thin bony osseous spiral lamina wall and into the auditory nerve [36]. Although some dispersal of transplanted cells is likely to be necessary for effective replacement of ANs, extensive dispersal has the potential disadvantage of transporting the cells to regions distal to their target site. In addition, the effect of the surgical procedure on the endogenous population of neurons and the tissue response in the cochlea are also important to evaluate when considering these potential therapies. Whilst these studies are promising, arguably the most important feature of an effective cell replacement strategy for AN replacement is whether new stem cell-derived neurons are electrophysiologically functional *in vivo* and capable of normal integration into existing structures and circuits in the brainstem. Given the precise cochleotopic organisation of the cochlear nucleus and specificity of synaptic connections within this brainstem structure, this may prove the ultimate challenge. In addition, there are no reports to date detailing the successful growth of stem cell-derived processes through the glia limitans and into the cochlear nucleus to synapse on second order neurons in the brainstem. The central growth of stem cell-derived neural processes will be an essential hurdle to overcome for combined stem cell-cochlear implant therapy, given that this neural circuit must be operational in order for the cochlear implant to effectively relay sound information to the brain.

3. Key morphological and biochemical features of stem cells for replacing the ANs

In developing a cell replacement therapy for SNHL, stem cells must be capable of differentiation into an appropriate neural subtype, presumably the type I ANs which comprise the majority of primary afferent neurons in the cochlea (for recent review refer to [62]). Specifically, this subtype must possess unique electrophysiological properties, such that they are capable of relaying pitch and timing cues necessary for speech perception with speed and precision (see further discussion below). Ideally, they would also need to be derived from human sources for use in the clinic. There are two choices of human stem cell types which can be generally classified into either embryonic or adult subtypes, and both have benefits which warrant further consideration. Whilst, human embryonic stem cells have a broad differentiation repertoire, they may be difficult to patient-match for transplantation purposes, and are surrounded by ethical controversies as they are derived from embryonic sources. Conversely, adult stem cells can be patient-matched and avoid the controversial use of embryos for this research, but are believed to have a more restricted differentiation potential which may prevent them being successfully differentiated into the subtype required for cochlear stem cell therapy. With the relatively recent discovery of induced pluripotent stem cells [63], it may be possible to combine the best characteristics of both embryonic and adult stem cell types, meaning the potential for patient-matched cells capable of multi-lineage differentiation. Clearly, there is still a great deal of work to do to investigate their

full potential for treating human disease, including cochlear rehabilitation ([48,52] as recently reviewed by [64]).

3.1 Differentiation of stem cells into an auditory neural phenotype

Whilst neural differentiation of human embryonic stem cells into neurons has been widely described [65–72], differentiation into defined lineages is still a major challenge. The differentiation of primary ANs is no exception and only a limited number of studies have reported the differentiation of stem cells toward an auditory neural-like lineage [22,24,45]. Of these studies, just one has used a human stem cell line [22]. Measured progress on this front can be explained in part by the fact that there is no single specific marker which characterises this neuronal subtype, and in part due to more general difficulties in the stem cells field including the generation of a homogenous population of differentiated cells from cells which innately produce almost all cell types of the body.

To direct the differentiation of stem cells toward an auditory neural phenotype, one may consider the developmental stages of inner ear formation in the embryo as a starting point. The development of neurosensory progenitors from the otic placode is thought to follow the same molecular principles of brain development, including the combined interaction of diffusible signals including fibroblast growth factors (3, 8 and 10), Shh, Wnts, and bone morphogenetic proteins [73–75]. The interaction between these four factors produces a patterning gradient which induces target cells into otic placode derivatives [73]. The primary ANs of the mammalian cochlea arise from the medial ectoderm of the otic placode [76], where they express *Eya1* [77,78], *Six1* [78], Pax2 [79–81] and Islet1 [81]. These neurons then undergo sequential expression of *neurogenin 1*, *NeuroD*, Brn3a and GATA3, before delaminating from the otocyst and comprising the ANs of the inner ear [82,83]. Mature ANs are critically dependent upon brain-derived neurotrophic factor (BDNF) and neurotrophin 3 (NT3) for ongoing survival *in vitro* [84–86] and *in vivo* [87–90], and express the tyrosine receptor kinases TrkB and TrkC respectively [91–93], which bind these neurotrophins. In the absence of a single marker for determining AN phenotype, scientists have used combinations of soluble molecules (fibroblast growth factors, bone morphogenetic proteins) and growth factors (BDNF, NT3, and others) expressed during auditory development to try and direct stem cells toward an auditory neural fate. They then probe for expression of the above-mentioned proteins known to be expressed during differentiation toward an AN *in situ*. Particularly promising assays have included those producing a population of GATA3-positive human stem cell-derived neurons [22], and mouse embryonic stem cells differentiated into the correct glutamatergic (VGLUT1/2 positive) phenotype [45].

3.2 Innervation and synapse formation of stem cell-derived neurons with endogenous cochlear tissues

In addition to generating an appropriate neural subtype for cell replacement, another important feature for the clinical application of stem cells in the nervous system is their ability to innervate and synapse upon appropriate endogenous tissues. Whilst several studies have reported that stem cells are capable of innervating and forming pre-synaptic terminals upon peripheral [22,26,27] and central [94] auditory target tissues *in vitro*, there are as yet no reports of post-synaptic density formation between these cell and tissue types. Moreover, synaptic contacts between stem cell-derived neurons and peripheral and/or central target tissue *in vivo* are yet to be documented in the auditory system. Whilst two reports have demonstrated that stem cell-derived neural processes grew in an ordered and fasciculating manner from the cochlea modiolus toward the peripheral hair cells, no synapses were observed between stem cells and the peripheral target tissue [22,35]. In addition, only a limited amount of central neurite outgrowth was reported in one of the studies [22]. If stem cell-derived neurons could be encouraged to extend processes centrally and into the cochlear

nucleus, then there is evidence to suggest that they may form synapses with endogenous tissue. This was illustrated using immuno-electron microscopy techniques following the transplantation of human embryonic stem cell-derived neurons into the neocortex [95]. Of course the detection of stem cell-derived synapses *in vivo* is particularly challenging, as is the tracing of stem cell-derived processes and indeed electrophysiological recordings from transplanted stem cells directly. In consideration of this, *in vitro* studies are useful in determining proof-of-principal concepts when it comes to the ability to extend processes toward peripheral and central targets, make synapses on the appropriate tissues and examine whether these are new circuits are electrophysiologically functional.

4. Key electrophysiological properties of stem cells for effective AN replacement

Whilst promising that stem cell-derived neurons have been observed to make synapses on appropriate auditory tissues types *in vitro* [22,26,27,94], the functionality of these new synapses remains to be determined, and this can only be thoroughly demonstrated using electrophysiology. The primary ANs of the cochlea are glutamatergic and capable of firing at high rates. The spike rate to acoustic stimulation (in the normal hearing cochlea) is typically in the order of 200–400 spikes/s [96,97], however this is much higher in response to electrical stimulation (in normal hearing or acutely deafened cochlea) at up to 1000 spikes/s over short periods [2,97]. This raises important considerations if stem cell-derived neurons are to be used in a cell replacement therapy, including: are stem cell-derived neurons electrically active; do they possess an appropriate compliment of ion channels; can they faithfully transmit electrical signals to the target neurons in the brain; and will they be able to respond to the high stimulus rate provided by the cochlear implant?

A detailed picture of the activity of the endogenous primary ANs, and the ion channels that underlie their activity, is emerging from the many *in vitro* studies undertaken over the last two decades [98–105]. Characterised by their response to a sustained injection of depolarising current, the *in vitro* firing behaviour of primary ANs has been categorised primarily on the basis of firing adaptation [102,106]. Rapidly-adapting responses are defined by a smaller number of action potentials (typically 1 or 2, and no more than 6) fired during stimulation, and limited to the beginning of the depolarising pulse. This phenotype is the predominant profile within the neural population, and across studies. Alternatively, slowly-adapting responses are denoted by action potentials fired throughout the stimulus. Although a slowly adapting response has been observed in a portion of auditory neurons derived from the apical portion of the cochlea [98,102] this represents only a small subset of the overall neural population. Though to date this has only been reported in murine neurons [98,102,106,107] following a number of days grown in culture [107]. Recordings from acutely-dissociated neurons and slice cultures exhibit a consistent rapidly-adapting profile [99–101,105].

The ion channels responsible for shaping the activity of ANs are summarised in Table 2. Key amongst these are members of the tetrodotoxin-sensitive voltage-gated sodium channel (Na_v) family that are necessary for the activation of action potentials. A large number of voltage-gated potassium channels (K_v) have also been identified, with their influence extending across many physiological features including action potential threshold, latency, duration and frequency. The rapidly-adapting firing profile that dominates within the AN population is specifically linked with high expression of K_v1.1 and K_v3.1 channels. As a low-voltage activated channel, K_v1.1 contributes to an increase in firing threshold and by mediating a slowly-inactivating current, limits repetitive firing. Alternatively K_v3.1 is activated at higher voltages (such as those reached during an action potential) and is associated with a reduction in action potential duration. The presence of a transient A-type

potassium current in rapidly-adapting ANs [100], whilst still somewhat contentious [see 108], is supported by the expression of channel subunits $K_{V1.4}$, 3.4, 4.2 and 4.3 in ANs. The A-type current has been associated with an increase in firing threshold and rapid repolarisation of the membrane following an action potential.

In addition to these core sodium and potassium channels, ANs also express a number of other channels that each play a pivotal role in shaping neural output including calcium-dependent potassium (K_{Ca}) channels, voltage-dependent calcium (Ca_V) channels and hyperpolarisation-activated cyclic nucleotide-gated (HCN) channels. The role of calcium channels and the calcium-dependent potassium channels are linked, and blockade of either leads to an increase in action potential latency [98,109]. In addition, high-voltage activated calcium channels contribute to the duration of action potentials [109], whilst the large-conductance calcium-activated potassium (BK_{Ca}) current has been shown to limit firing frequency [98]. Lastly, the hyperpolarisation-activated mixed-cation current (I_h) mediated by HCN channels is prevalent throughout the population of primary ANs [99,103,116] and has been implicated in setting resting membrane properties, contributing to firing threshold, as well as limiting the duration of excitatory postsynaptic potentials [115].

Together, this combination of channels provide ANs with the ability to follow high rates of stimulation with temporal acuity. As demonstrated in whole-cell recordings [101], individual primary ANs can reliably entrain to 50 Hz stimulation (20 ms interval between biphasic pulses). At 100 Hz (10 ms interval) and 200 Hz (5 ms interval) firing reliability decreases to around 70% and 40% respectively. Our own recordings of cultured rat primary ANs displaying a rapidly-adapting profile (Fig 2) show a similar outcome with monophasic pulses. In the first example (Fig. 2A), firing of an AN can reliably entrain to 100 and 200 Hz stimuli. Whilst in a second AN (Fig. 2B) firing is consistent at 20 Hz (100 ms interval between pulses), but falls below 100% when the pulse interval decreases to 50 ms (67 Hz).

To replace the degenerating endogenous primary afferent neurons in the most functionally meaningful manner, stem cell-derived neurons might be expected to share the same (or similar) physiological phenotypes. Whilst many studies have explored electrical activity in stem cells, investigations of the physiological properties of stem cell-derived neurons directed towards an auditory fate have been limited. Martinez-Monedero and colleagues [81], examining the electrophysiological activity of neurons derived from inner ear stem cells of mice, demonstrated that all neurons displayed a high voltage-activated potassium current, but few (7%) exhibit a low-voltage activated potassium current. Tetrodotoxin-sensitive sodium currents were limited to less than half of the cells tested, and of those, only 40% evoked action potentials. More recently, glutamatergic neurons derived from mouse embryonic stem cells over-expressing *neurogenin 1* [120] were also examined for the ability to replace sensory neurons. *Neurog1*-induced cells were excitable, firing action potentials in a rapidly-adapting pattern as per ANs. Action potentials were tetrodotoxin-sensitive, and cells expressed sodium channel subunit $Na_V1.6$. Further, calcium currents were identified, as well as both high-voltage activated sustained potassium current and transient A-type current. Pharmacological analysis suggested K_V2 and $K_V3.1$ underlie the high voltage current, whilst $K_V1.4$ or K_V4 was nominated to give rise to A-type activity. Importantly, Chen and colleagues[25*] provided the first electrophysiological investigation of human fetal auditory stem cells. The study revealed the development of neuronal-like qualities in many of these cells including expression of a fast inward sodium current and outward potassium currents, but indicated an absence of calcium and hyperpolarisation-activated currents.

Most recently, we have shown that human embryonic stem cell-derived auditory-like neurons are electrically active, firing action potentials in a rapidly-adapting fashion (Fig.

2C–D) in a similar manner to those observed in cultured primary ANs under the same recording conditions (Fig. 2A–B). Stem cell-derived neurons also demonstrated the presence of tetrodotoxin-sensitive sodium currents and sustained potassium currents (Fig. 2F–H). Yet, action potentials were broader (half-width of 2.6 ms, $n=20$) in the stem cell-derived neurons than those recorded from primary ANs (0.8 ms, $n=82$; Fig. 2E). This activity was also accompanied by an increase in action potential latency at firing threshold (35.9 ms) as well as in response to supra-threshold stimuli (5.6 ms) compared to ANs (12.7 ms and 2.5 ms, respectively). Further, whilst I_h is consistently observed in ANs, this hyperpolarisation-activated current was absent from the human embryonic stem cell-derived neurons. These findings suggest that whilst stem cell-derived neurons display the key electrophysiological features (rapidly-adapting firing; tetrodotoxin-sensitive sodium currents; sustained potassium currents) of the ANs they are designed to replace, they do not yet possess all the attributes of post-natal ANs. Rather, they closely resemble the activity of the embryonic ANs [121]. As shown by the response of the stem cell-derived neurons to pulse trains at 20 and 67 Hz (Fig. 2C–D), firing entrainment to moderate rates of stimulation is limited by the relatively broad action potentials. Differentiation of stem cell-derived neurons capable of shorter latency, and brief action potentials through expression of additional ion channel families (such as Ca_v , K_{Ca} and HCN) will be necessary if they are to emulate the activity of ANs.

5. Expert opinion

There is often a prolonged delay between the loss of hearing and the implementation of treatment, resulting in progressive degeneration of the auditory nerve. Whilst degeneration can be halted by the infusion of exogenous neurotrophin into the cochlea, this survival effect is lost following the cessation of treatment, and cochlear implants remain the only routinely used clinical option for the treatment of SNHL. Stem cell therapy offers the potential for nerve replacement in the severely deafened cochlea by transplantation of stem cell-derived neurons. The success of such therapy in the future will critically depend upon whether these stem cell-derived neurons are functional and can project central axons to the cochlear nucleus that establish functional synapses in a cochleotopic manner. If successful, such a therapy will be important for hair cell and nerve regeneration studies, considering that the success of both therapies relies upon a critical number of surviving primary ANs following deafness. It may also benefit patients with auditory neuropathy, given that this cohort of individuals can derive benefits from cochlear implantation [122,123].

Whilst challenging, the surgical approach to deliver stem cell-derived neurons into the cochlear modiolus seems feasible since this is now being routinely used in gerbils and other small rodent models. By comparison the length of the scala tympani in small rodents is 4.55 mm (mice), 9.20 mm (gerbil) and 7.24 mm (rat), and 28.46 mm (human), [124]. It is also promising to note that stem cells survive in the deafened cochlea environment for periods of at least three months, however longer-term studies are required in order to ensure that these cells continue to thrive indefinitely; presumably integrating into the host's existing vasculature system where they can metabolise effectively. To the best of our knowledge, this has not yet been demonstrated and may require investigation at the electron microscopy level to thoroughly do so. It will also be necessary to provide patient-matched cells or alternatively, appropriate immunosuppression, in order to ensure the longevity of cell transplants in the cochlea, and this concept has been reviewed in detail elsewhere [29,64]. It should be noted that despite encouraging advances in human embryonic stem cell differentiation and transplantation, a major difficulty remains to be addressed before stem cell therapies are clinically viable. That is, the mismatch of major human leukocyte antigens between the donor cells and the host will result in their eventual immunorejection by the host. Although chronic immunosuppression can protect against immunorejection of donor

cells, it is not without its own significant drawbacks, including cancer and infection [125]. Even the recently discovered induced pluripotent stem cells [63], which were thought to be a source of autologous cells for transplantation [126], have since been reported as showing genomic instability, abnormalities in their epigenetics, immunogenicity and capable of teratoma formation [127,128]. Collectively, these factors affect the entire cell therapy field and are perhaps the most significant obstacle to the clinical application of stem cells. A detailed report of the current challenges and recent progress in overcoming these, has been recently reviewed by [127].

A perfect genotypic copy of an endogenous AN seems to be the ultimate goal of stem cell cochlear therapy (ie. for reconnection with regenerated hair cells), this may not be necessary in order to achieve improved outcomes with a cochlear implant. Key biochemical attributes of stem cell-derived neurons for combined treatment with electrical stimulation include differentiation into an excitatory (glutamatergic [45]) subtype, such that these new neurons (if functionally active) could readily integrate into the established auditory system. In addition, the expression of TrkB/C would likely confer survival advantages in the auditory system as new neurons would benefit from both the residual expression of BDNF/NT3 from the peripheral and/or central auditory system and/or emerging neurotrophin delivery strategies [11,28,129] and cochlear implant designs [130]; the latter of which is aimed at preserving both the residual endogenous population, and any new neurons in the cochlea. Delivery of exogenous neurotrophins may be further harnessed to alter the activity of stem cell-derived neurons, as BDNF and NT3 can manipulate firing patterns through modulation of channel expression [106,131]. Interestingly, new generation implants and advances in stem cell differentiation may make it possible in future to engineer neurons from stem cells which are far superior to ANs in terms of their ability to receive and respond to high rate stimulation. This is a focus of current investigations in our laboratory (Fig. 2), and ability to follow high-rate stimulation may be improved following exposure to electrical stimulation *in vitro* [132] and/or *in vivo* [133]. Moreover, methods to improve the dynamic range of the stem cell-derived neurons (and other performance characteristics of prostheses) may result in better outcomes for cochlear implant recipients. Key to the successful integration of transplanted stem cell-derived neurons, is their stage of differentiation at the time of delivery. The less differentiated the cells, the more potential they are observed to have in terms of their migration and distribution throughout the cochlea [145]. Human neural progenitors have been shown to form synapses on sensory hair cells *in vitro* [22,27] and survive for up to three months *in vivo*, extending processes toward residual hair cells in the denervated cochlea [22]. It remains to be elucidated whether these progenitors are capable of making functional central connections *in vivo*, but they have been observed to do so with cochlear nucleus slices *in vitro* [94,146].

Electrical stimulation *in vitro* and/or *in vivo* may have multiple positive effects on the differentiation and integration of appropriate neural subtypes for AN replacement including enhanced firing properties and improved survival, neurite outgrowth and synapse formation. We postulate that electrical stimulation may act to mimic the spontaneous activity present in the developing auditory system *in situ*, and this spontaneous activity in developing auditory nerve fibres is considered essential for the survival of target neurons in the cochlear nucleus [82]. It is also suggested that spontaneous firing may assist in directing the formation of specific synaptic connections and in activating ANs during embryogenesis [134,135]. Similarly, electrical stimulation has been shown to promote survival of ANs *in vitro* [136,137] and *in vivo* [138], and additionally promotes the differentiation of stem cells into neural phenotypes *in vitro* [139]. As such, electrically stimulating these new (stem cell-derived) neurons may enhance their ability to respond and adapt to high rates of stimulation, particularly if provided at the critical times during their differentiation and development.

Taken collectively, these data strongly support the use of electrical stimulation in promoting functional reconnection of stem cell-derived neurons in the central auditory system.

Given the development of a fully-implantable small rodent cochlear implant electrode array [133] it is now possible to both electrically stimulate transplanted stem cell-derived neurons and also measure their function *in vivo*, using electrically evoked auditory brainstem responses. The auditory system is one of the few neural systems in which such hypotheses can be readily tested. Using this technology, stem cells can be both electrically stimulated *in vivo* at behaviourally appropriate stimulus levels, and any improvements in function quantified over time by comparing differences in activation thresholds. Given that normal synaptic activity can be recovered following chronic electrical stimulation in congenitally deaf cats [140], the *in vivo* depolarisation of stem cell-derived neurons may assist in synapse formation between stem cells and endogenous neurons. It is also conceivable that, even with a rudimentary neural scaffold remaining, stem cell-derived neurons may have a template upon which to extend processes into the correct areas of the brainstem. Given that the cochlear implant will depolarise both exogenous and endogenous neurons, this may provide additional guidance cues for cochleotopic reconnection in the cochlear nucleus. Importantly, if neurons reconnect in a sporadic manner in the cochlear nucleus, central processing may be altered and normal implant function disturbed. This will be a critical obstacle to overcome in the clinical translation of this therapy.

As detailed throughout this review, enhanced outgrowth of stem cell-derived processes is fundamental to facilitating functional connection to central targets. Therefore, observations that electrical stimulation can significantly enhance neurite outgrowth when combined with neurotrophin application [141] are particularly encouraging. These findings are further supported by recent studies in the peripheral nervous system, which illustrate the positive effect of electrical stimulation on the accelerated regrowth of endogenous sensory axons [142]. If combined with the application of repulsive guidance molecule inhibitors, which have been reported to improve axon regrowth through glial scar tissue after spinal cord injury [143,144], growth through the glia limitans is promising. The delivery of these molecules could conceivably occur at the same time as stem cells were transplanted, and need only last long enough to allow for new processes to extend and synapse on central targets. In future, if stem cell-derived neurons can be encouraged to make functional and cochleotopic central connections using the combined approaches above, there may be a broader population of patients that can derive benefits from a cochlear implant. Importantly, research in this field has the potential to inform other emerging therapies which combine cell transplantation with electrical stimulation of tissue, including cardiac pacemakers with stem cell-derived cardiac tissue for improved heart function [147–149], or retinal prostheses combined with stem cell-derived retinal tissues to restore sight [150,151].

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References

1. Fritsch B, Farinas I, Reichardt LF. Lack of neurotrophin 3 causes losses of both classes of spiral ganglion neurons in the cochlea in a region-specific fashion. *J Neurosci*. 1997; 17:6213–6225. [PubMed: 9236232]

2. Shepherd RK, Javel E. Electrical stimulation of the auditory nerve. I. Correlation of physiological responses with cochlear status. *Hear Res.* 1997; 108:112–144. [PubMed: 9213127]
3. Hardie NA, Shepherd RK. Sensorineural hearing loss during development: morphological and physiological response of the cochlear and auditory brainstem. *Hear Res.* 1999; 128:147–165. [PubMed: 10082295]
4. Leake PA, Hradek GT. Cochlear pathology of long term neomycin induced deafness in cats. *Hear Res.* 1988; 33:11–33. [PubMed: 3372368]
5. Staecker H, Kopke R, Malgrange B, et al. NT-3 and/or BDNF therapy prevents loss of auditory neurons following loss of hair cells. *Neuroreport.* 1996; 7:889–894. [PubMed: 8724667]
6. Gillespie LN, Clark GM, Marzella PL. Delayed neurotrophin treatment supports auditory neuron survival in deaf guinea pigs. *Neuroreport.* 2004; 15:1121–1125. [PubMed: 15129158]
7. Richardson RT, O'Leary S, Wise A, et al. A single dose of neurotrophin-3 to the cochlea surrounds spiral ganglion neurons and provides trophic support. *Hear Res.* 2005; 204:37–47. [PubMed: 15925190]
8. Miller JM, Chi DH, O'Keefe LJ, et al. Neurotrophins can enhance spiral ganglion cell survival after inner hair cell loss. *Int J Dev Neurosci.* 1997; 15:631–643. [PubMed: 9263039]
9. Agterberg MJ, Versnel H, van Dijk LM, et al. Enhanced survival of spiral ganglion cells after cessation of treatment with brain-derived neurotrophic factor in deafened guinea pigs. *J Assoc Res Otolaryngol.* 2009; 10:355–367. [PubMed: 19365690]
10. Gillespie LN, Clark GM, Bartlett PF, et al. BDNF-induced survival of auditory neurons in vivo: cessation of treatment leads to accelerated loss of survival effects. *J Neurosci Res.* 2003; 71:785–790. [PubMed: 12605404]
11. Pettingill LN, Wise AK, Geaney MS, et al. Enhanced auditory neuron survival following cell-based BDNF treatment in the deaf guinea pig. *PLoS One.* 2011; 6:e18733.
12. Gillespie LN, Shepherd RK. Clinical application of neurotrophic factors: the potential for primary auditory neuron protection. *Eur J Neurosci.* 2005; 22:2123–2133. [PubMed: 16262651]
13. Linthicum FH Jr, Fayad JN. Spiral ganglion cell loss is unrelated to segmental cochlear sensory system degeneration in humans. *Otol Neurotol.* 2009; 30:418–422. [PubMed: 19326501]
14. Teufert KB, Linthicum FH Jr, Connell SS. The effect of organ of corti loss on ganglion cell survival in humans. *Otol Neurotol.* 2006; 27:1146–1151. [PubMed: 16885783]
15. Li H, Corrales CE, Edge A, et al. Stem cells as therapy for hearing loss. *Trends Mol Med.* 2004; 10:309–315. [PubMed: 15242678]
16. Li H, Roblin G, Liu H, et al. Generation of hair cells by stepwise differentiation of embryonic stem cells. *Proc Natl Acad Sci.* 2003; 100:13495–13500. [PubMed: 14593207]
17. Oshima K, Shin K, Diensthuber M, et al. Mechanosensitive hair cell-like cells from embryonic and induced pluripotent stem cells. *Cell.* 2010; 141:704–716. [PubMed: 20478259] First report of electrically active hair cell-like cells from pluripotent stem cells
18. Starr A, Picton TW, Sininger Y, et al. Auditory neuropathy. *Brain.* 1996; 119(Pt 3):741–753. [PubMed: 8673487]
19. Rance G, Beer DE, Cone-Wesson B, et al. Clinical findings for a group of infants and young children with auditory neuropathy. *Ear Hear.* 1999; 20:238–252. [PubMed: 10386850]
20. Shibata SB, Budenz CL, Bowling SA, et al. Nerve maintenance and regeneration in the damaged cochlea. *Hear Res.* 2011; 281:56–64. [PubMed: 21596129]
21. Zeng FG, Oba S, Garde S, et al. Temporal and speech processing deficits in auditory neuropathy. *Neuroreport.* 1999; 10:3429–3435. [PubMed: 10599857]
22. Shi F, Corrales CE, Liberman MC, et al. BMP4 induction of sensory neurons from human embryonic stem cells and reinnervation of sensory epithelium. *Eur J Neurosci.* 2007; 26:3016–3023. [PubMed: 18005071] *In vivo* study illustrating the peripheral outgrowth of human embryonic stem cell-derived neural progenitors toward the organ of Corti in the denervated cochlea.
23. Coleman B, Fallon JB, Pettingill LN, et al. Auditory hair cell explant co-cultures promote the differentiation of stem cells into bipolar neurons. *Exp Cell Res.* 2007; 313:232–243. [PubMed: 17112512]

24. Nayagam BA, Minter RL. A Comparison of in vitro treatments for directing stem cells toward a sensory neural fate. *American Journal of Otolaryngology*. 2012; 33:37–46. [PubMed: 21439680]
25. Chen W, Johnson SL, Marcotti W, et al. Human fetal auditory stem cells can be expanded in vitro and differentiate into functional auditory neurons and hair cell-like cells. *Stem Cells*. 2009; 27:1196–1204. [PubMed: 19418454] Provided the first electrophysiological investigation and demonstration of the functional potential of human fetal auditory stem cells
26. Matsumoto M, Nakagawa T, Kojima K, et al. Potential of embryonic stem cell-derived neurons for synapse formation with auditory hair cells. *J Neurosci Res*. 2008; 86:3075–3085. [PubMed: 18543340]
27. Nayagam, BA.; Edge, A.; Dottori, M. Proceedings of the Association for Research in Otolaryngology. Baltimore, MD: 2012. Stem Cell-Derived Sensory Progenitors can Innervate the Early Post-Natal Sensory Epithelium In Vitro; p. 8
28. Wise AK, Fallon JB, Neil AJ, et al. Combining cell-based therapies and neural prostheses to promote neural survival. *Neurotherapeutics*. 2011; 8:774–787. [PubMed: 21904788]
29. Coleman B, de Silva MG, Shepherd RK. Concise review: the potential of stem cells for auditory neuron generation and replacement. *Stem Cells*. 2007; 25:2685–2694. [PubMed: 17656641]
30. Coleman B, Hardman J, Coco A, et al. Fate of embryonic stem cells transplanted into the deafened mammalian cochlea. *Cell Transplant*. 2006; 15:369–380. [PubMed: 16970279]
31. Hu Z, Ulfendahl M, Olivius NP. Survival of neuronal tissue following xenograft implantation into the adult rat inner ear. *Exp Neurol*. 2004; 185:7–14. [PubMed: 14697314]
32. Olivius P, Alexandrov L, Miller J, et al. Allografted fetal dorsal root ganglion neuronal survival in the guinea pig cochlea. *Brain Res*. 2003; 979:1–6. [PubMed: 12850564]
33. Okano T, Nakagawa T, Endo T, et al. Engraftment of embryonic stem cell-derived neurons into the cochlear modiolus. *Neuroreport*. 2005; 16:1919–1922. [PubMed: 16272879]
34. Regala C, Duan M, Zou J, et al. Xenografted fetal dorsal root ganglion, embryonic stem cell and adult neural stem cell survival following implantation into the adult vestibulocochlear nerve. *Exp Neurol*. 2005; 193:326–333. [PubMed: 15869935]
35. Corrales CE, Pan L, Li H, et al. Engraftment and differentiation of embryonic stem cell-derived neural progenitor cells in the cochlear nerve trunk: Growth of processes into the organ of corti. *J Neurobiol*. 2006; 66:1489–5000. [PubMed: 17013931] *In vivo* study demonstrating the peripheral outgrowth of mouse embryonic stem cell-derived neural progenitors toward the organ of Corti in the denervated cochlea
36. Nayagam BA, Backhouse S, Cimenkaya C, et al. Hydrogel limits stem cell dispersal in the deaf cochlea: implications for cochlear implants. *Journal of Neural Engineering*. 2012:1–14. *In press*.
37. Hu Z, Andang M, Ni D, et al. Neural cogaft stimulates the survival and differentiation of embryonic stem cells in the adult mammalian auditory system. *Brain Res*. 2005; 1051:137–144. [PubMed: 15996640]
38. Hu Z, Wei D, Johansson CB, et al. Survival and neural differentiation of adult neural stem cells transplanted into the mature inner ear. *Exp Cell Res*. 2005; 302:40–47. [PubMed: 15541724]
39. Hu Z, Ulfendahl M, Olivius NP. NGF stimulates extensive neurite outgrowth from implanted dorsal root ganglion neurons following transplantation into the adult rat inner ear. *Neurobiol Dis*. 2005; 18:184–192. [PubMed: 15649709]
40. Matsuoka AJ, Kondo T, Miyamoto RT, et al. In vivo and in vitro characterization of bone marrow-derived stem cells in the cochlea. *Laryngoscope*. 2006; 116:1363–1367. [PubMed: 16885736]
41. Matsuoka AJ, Kondo T, Miyamoto RT, et al. Enhanced survival of bone-marrow-derived pluripotent stem cells in an animal model of auditory neuropathy. *Laryngoscope*. 2007; 117:1629–1635. [PubMed: 17632425]
42. Parker MA, Corliss DA, Gray B, et al. Neural stem cells injected into the sound-damaged cochlea migrate throughout the cochlea and express markers of hair cells, supporting cells, and spiral ganglion cells. *Hear Res*. 2007; 232:29–43. [PubMed: 17659854]
43. Altschuler RA, O'Shea KS, Miller JM. Stem cell transplantation for auditory nerve replacement. *Hear Res*. 2008; 242:110–116. [PubMed: 18585449]

44. Lang H, Schulte BA, Goddard JC, et al. Transplantation of mouse embryonic stem cells into the cochlea of an auditory-neuropathy animal model: effects of timing after injury. *J Assoc Res Otolaryngol*. 2008; 9:225–240. [PubMed: 18449604]
45. Reyes JH, O'Shea KS, Wys NL, et al. Glutamatergic neuronal differentiation of mouse embryonic stem cells after transient expression of neurogenin 1 and treatment with BDNF and GDNF: in vitro and in vivo studies. *J Neurosci*. 2008; 28:12622–12631. [PubMed: 19036956] First *in vivo* demonstration of differentiation of embryonic stem cells toward a glutamatergic phenotype following transplantation into the deaf cochlea
46. Fu Y, Wang S, Liu Y, et al. Study on neural stem cell transplantation into natural rat cochlea via round window. *Am J Otolaryngol*. 2009; 30:8–16. [PubMed: 19027507]
47. Hu Z, Ulfendahl M, Prieskorn DM, et al. Functional evaluation of a cell replacement therapy in the inner ear. *Otol Neurotol*. 2009; 30:551–558. [PubMed: 19395986]
48. Nishimura K, Nakagawa T, Ono K, et al. Transplantation of mouse induced pluripotent stem cells into the cochlea. *Neuroreport*. 2009; 20:1250–1254. [PubMed: 19625987]
49. Sullivan JM, Cohen MA, Pandit SR, et al. Effect of epithelial stem cell transplantation on noise-induced hearing loss in adult mice. *Neurobiol Dis*. 2011; 41:552–559. [PubMed: 21059389]
50. Pandit SR, Sullivan JM, Egger V, et al. Functional effects of adult human olfactory stem cells on early-onset sensorineural hearing loss. *Stem Cells*. 2011; 29:670–677. [PubMed: 21312317]
51. Cho YB, Cho HH, Jang S, et al. Transplantation of neural differentiated human mesenchymal stem cells into the cochlea of an auditory-neuropathy guinea pig model. *J Korean Med Sci*. 2011; 26:492–498. [PubMed: 21468255]
52. Nishimura K, Nakagawa T, Sakamoto T, et al. Fates of murine pluripotent stem cell-derived neural progenitors following transplantation into mouse cochleae. *Cell Transplant*. 2012
53. Hu Z, Ulfendahl M, Olivius NP. Central migration of neuronal tissue and embryonic stem cells following transplantation along the adult auditory nerve. *Brain Res*. 2004; 1026:68–73. [PubMed: 15476698]
54. Naito Y, Nakamura T, Nakagawa T, et al. Transplantation of bone marrow stromal cells into the cochlea of chinchillas. *Neuroreport*. 2004; 15:1–4. [PubMed: 15106820]
55. Tamura T, Nakagawa T, Iguchi F, et al. Transplantation of neural stem cells into the modiolus of mouse cochleae injured by cisplatin. *Acta Otolaryngol Suppl*. 2004:65–68. [PubMed: 15078082]
56. Ogita H, Nakagawa T, Sakamoto T, et al. Transplantation of bone marrow-derived neurospheres into guinea pig cochlea. *Laryngoscope*. 2010; 120:576–581. [PubMed: 20058318]
57. Sekiya T, Kojima K, Matsumoto M, et al. Cell transplantation to the auditory nerve and cochlear duct. *Exp Neurol*. 2005; 198:12–24. [PubMed: 16376874]
58. Ahn KS, Jeon SJ, Jung JY, et al. Isolation of embryonic stem cells from enhanced green fluorescent protein-transgenic mouse and their survival in the cochlea after allotransplantation. *Cytotherapy*. 2008; 10:759–769. [PubMed: 18985482]
59. Praetorius M, Vicario I, Schimmang T. Efficient transfer of embryonic stem cells into the cochlea via a non-invasive vestibular route. *Acta Otolaryngol*. 2008; 128:720–723. [PubMed: 18568511]
60. Iguchi F, Nakagawa T, Tateya I, et al. Surgical techniques for cell transplantation into the mouse cochlea. *Acta Otolaryngol Suppl*. 2004:43–47. [PubMed: 15078077]
61. Revoltella RP, Papini S, Rosellini A, et al. Cochlear repair by transplantation of human cord blood CD133+ cells to nod-scid mice made deaf with kanamycin and noise. *Cell Transplant*. 2008; 17:665–678. [PubMed: 18819255]
62. Nayagam BA, Muniak MA, Ryugo DK. The spiral ganglion: connecting the peripheral and central auditory nervous systems. *Hearing Research*. 2011; 278:2–20. [PubMed: 21530629] Relevant detailed review on the anatomical and biochemical characteristics of auditory neurons
63. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006; 126:663–676. [PubMed: 16904174] Seminal reference detailing the discovery of induced pluripotent stem cells
64. Gunewardene N, Dottori M, Nayagam BA. The Convergence of Cochlear Implantation with Induced Pluripotent Stem Cell Therapy. *Stem Cell Rev*. 2011

65. Gerrard L, Rodgers L, Cui W. Differentiation of human embryonic stem cells to neural lineages in adherent culture by blocking bone morphogenetic protein signaling. *Stem Cells*. 2005; 23:1234–1241. [PubMed: 16002783]
66. Carpenter MK, Inokuma MS, Denham J, et al. Enrichment of neurons and neural precursors from human embryonic stem cells. *Exp Neurol*. 2001; 172:383–397. [PubMed: 11716562]
67. Ladewig J, Koch P, Endl E, et al. Lineage selection of functional and cryopreservable human embryonic stem cell-derived neurons. *Stem Cells*. 2008; 26:1705–1712. [PubMed: 18420830]
68. Park S, Lee KS, Lee YJ, et al. Generation of dopaminergic neurons in vitro from human embryonic stem cells treated with neurotrophic factors. *Neurosci Lett*. 2004; 359:99–103. [PubMed: 15050721]
69. Reubinoff BE, Itsykson P, Turetsky T, et al. Neural progenitors from human embryonic stem cells. *Nat Biotechnol*. 2001; 19:1134–1140. [PubMed: 11731782]
70. Schuldiner M, Eiges R, Eden A, et al. Induced neuronal differentiation of human embryonic stem cells. *Brain Res*. 2001; 913:201–205. [PubMed: 11549388]
71. Schulz TC, Palmarini GM, Noggle SA, et al. Directed neuronal differentiation of human embryonic stem cells. *BMC Neurosci*. 2003; 4:27. [PubMed: 14572319]
72. Zhang SC, Wernig M, Duncan ID, et al. In vitro differentiation of transplantable neural precursors from human embryonic stem cells. *Nat Biotechnol*. 2001; 19:1129–1133. [PubMed: 11731781]
73. Fritzsch B, Eberl DF, Beisel KW. The role of bHLH genes in ear development and evolution: revisiting a 10-year-old hypothesis. *Cell Mol Life Sci*. 2010; 67:3089–3099. [PubMed: 20495996]
Provides a detailed summary of the factors influencing the differentiation of auditory neurons *in situ*
74. Ohyama T, Groves AK, Martin K. The first steps towards hearing: mechanisms of otic placode induction. *Int J Dev Biol*. 2007; 51:463–472. [PubMed: 17891709]
75. Fritzsch B, Beisel KW, Hansen LA. The molecular basis of neurosensory cell formation in ear development: a blueprint for hair cell and sensory neuron regeneration? *Bioessays*. 2006; 28:1181–1193. [PubMed: 17120192]
76. Rubel, EW. Ontogeny of structure and function in the vertebrate auditory system. In: Jacobsen, M., editor. *Handbook of sensory physiology*. New York: Springer-Verlag; 1978. p. 135-237.
77. Friedman RA, Makmura L, Biesiada E, et al. Eya1 acts upstream of Tbx1, Neurogenin 1, NeuroD and the neurotrophins BDNF and NT-3 during inner ear development. *Mech Dev*. 2005; 122:625–634. [PubMed: 15817220]
78. McMillan, EL.; Foate, AL.; Gebhardt, EK., et al. International Society for Stem Cell Research. San Francisco: 2010. Directed differentiation of mouse and human pluripotent stem cells generates otic progenitor and inner ear hair cells.
79. Torres M, Gomez-Pardo E, Gruss P. Pax2 contributes to inner ear patterning and optic nerve trajectory. *Development*. 1996; 122:3381–3391. [PubMed: 8951055]
80. Burton Q, Cole LK, Mulheisen M, et al. The role of Pax2 in mouse inner ear development. *Dev Biol*. 2004; 272:161–175. [PubMed: 15242798]
81. Martinez-Monedero R, Yi E, Oshima K, et al. Differentiation of inner ear stem cells to functional sensory neurons. *Dev Neurobiol*. 2008; 68:669–684. [PubMed: 18278797]
82. Rubel EW, Fritzsch B. Auditory system development: primary auditory neurons and their targets. *Ann Rev Neurosci*. 2002; 25:51–101. [PubMed: 12052904]
83. Fritzsch B. Development of inner ear afferent connections: forming primary neurons and connecting them to the developing sensory epithelia. *Brain Res Bull*. 2003; 60:423–433. [PubMed: 12787865]
84. Malgrange B, Lefebvre P, Van de Water TR, et al. Effects of neurotrophins on early auditory neurones in cell culture. *Neuroreport*. 1996; 7:913–917. [PubMed: 8724672]
85. Zheng JL, Gao WQ. Differential damage to auditory neurons and hair cells by ototoxins and neuroprotection by specific neurotrophins in rat cochlear organotypic cultures. *Eur J Neurosci*. 1996; 8:1897–1905. [PubMed: 8921280]
86. Marzella PL, Clark GM, Shepherd RK, et al. LIF potentiates the NT-3-mediated survival of spiral ganglia neurones in vitro. *Neuroreport*. 1997; 8:1641–1644. [PubMed: 9189906]

87. Pirvola U, Ylikoski J, Palgi J, et al. Brain-derived neurotrophic factor and neurotrophin 3 mRNAs in the peripheral target fields of developing inner ear ganglia. *Proc Natl Acad Sci.* 1992; 89:9915–9919. [PubMed: 1409719]
88. Ernfors P, Merlio JP, Persson H. Cells Expressing mRNA for Neurotrophins and their Receptors During Embryonic Rat Development. *Eur J Neurosci.* 1992; 4:1140–1158. [PubMed: 12106420]
89. Ylikoski J, Pirvola U, Moshnyakov M, et al. Expression patterns of neurotrophin and their receptor mRNAs in the rat inner ear. *Hear Res.* 1993; 65:69–78. [PubMed: 8080462]
90. Stankovic K, Rio C, Xia A, et al. Survival of adult spiral ganglion neurons requires erbB receptor signaling in the inner ear. *J Neurosci.* 2004; 24:8651–8661. [PubMed: 15470130]
91. Minichiello L, Piehl F, Vazquez E, et al. Differential effects of combined trk receptor mutations on dorsal root ganglion and inner ear sensory neurons. *Development.* 1995; 121:4067–4075. [PubMed: 8575307]
92. Schimmang T, Minichiello L, Vazquez E, et al. Developing inner ear sensory neurons require TrkB and TrkC receptors for innervation of their peripheral targets. *Development.* 1995; 121:3381–3391. [PubMed: 7588071]
93. Silos-Santiago I, Fagan AM, Garber M, et al. Severe sensory deficits but normal CNS development in newborn mice lacking TrkB and TrkC tyrosine protein kinase receptors. *Eur J Neurosci.* 1997; 9:2045–2056. [PubMed: 9421165]
94. Glavaski-Joksimovic A, Thonabulsombat C, Wendt M, et al. Morphological differentiation of tau-green fluorescent protein embryonic stem cells into neurons after co-culture with auditory brain stem slices. *Neuroscience.* 2009
95. Nasonkin I, Mahairaki V, Xu L, et al. Long-term, stable differentiation of human embryonic stem cell-derived neural precursors grafted into the adult mammalian neostriatum. *Stem Cells.* 2009; 27:2414–2426. [PubMed: 19609935]
96. Kiang, NY.; Watanabe, T.; Thomas, EC., et al. *Discharge Patterns of Single Fibers in the Cat's Auditory Nerve.* Cambridge, MA.: MIT Press; 1965.
97. Javel E, Viemeister NF. Stochastic properties of cat auditory nerve responses to electric and acoustic stimuli and application to intensity discrimination. *J Acoust Soc Am.* 2000; 107:908–921. [PubMed: 10687700]
98. Adamson CL, Reid MA, Mo ZL, et al. Firing features and potassium channel content of murine spiral ganglion neurons vary with cochlear location. *J Comp Neurol.* 2002; 447:331–350. [PubMed: 11992520]
99. Chen C. Hyperpolarization-activated current (I_h) in primary auditory neurons. *Hear Res.* 1997; 110:179–190. [PubMed: 9282900]
100. Jagger DJ, Housley GD. A-type potassium currents dominate repolarisation of neonatal rat primary auditory neurones in situ. *Neuroscience.* 2002; 109:169–182. [PubMed: 11784708]
101. Lin X. Action potentials and underlying voltage-dependent currents studied in cultured spiral ganglion neurons of the postnatal gerbil. *Hear Res.* 1997; 108:157–179. [PubMed: 9213129]
102. Mo ZL, Davis RL. Endogenous firing patterns of murine spiral ganglion neurons. *J Neurophysiol.* 1997; 77:1294–1305. [PubMed: 9084597]
103. Mo ZL, Davis RL. Heterogeneous voltage dependence of inward rectifier currents in spiral ganglion neurons. *J Neurophysiol.* 1997; 78:3019–3027. [PubMed: 9405521]
104. Santos-Sacchi J. Voltage-dependent ionic conductances of type I spiral ganglion cells from the guinea pig inner ear. *J Neurosci.* 1993; 13:3599–3611. [PubMed: 8393487]
105. Szabo ZS, Harasztosi CS, Sziklai I, et al. Ionic currents determining the membrane characteristics of type I spiral ganglion neurons of the guinea pig. *Eur J Neurosci.* 2002; 16:1887–1895. [PubMed: 12453052]
106. Adamson CL, Reid MA, Davis RL. Opposite actions of brain-derived neurotrophic factor and neurotrophin-3 on firing features and ion channel composition of murine spiral ganglion neurons. *J Neurosci.* 2002; 22:1385–1396. [PubMed: 11850465]
107. Sun W, Salvi RJ. Brain derived neurotrophic factor and neurotrophic factor 3 modulate neurotransmitter receptor expressions on developing spiral ganglion neurons. *Neuroscience.* 2009; 164:1854–1866. [PubMed: 19778585]

108. Rusznak Z, Szucs G. Spiral ganglion neurones: an overview of morphology, firing behaviour, ionic channels and function. *Pflugers Arch.* 2009; 457:1303–1325. [PubMed: 18777041]
109. Chen WC, Xue HZ, Hsu YL, et al. Complex distribution patterns of voltage-gated calcium channel alpha-subunits in the spiral ganglion. *Hear Res.* 2011; 278:52–68. [PubMed: 21281707]
110. Moore EJ, Hall DB, Narahashi T. Sodium and potassium currents of type I spiral ganglion cells from rat. *Acta Otolaryngol.* 1996; 116:552–560. [PubMed: 8831841]
111. Fryatt AG, Vial C, Mulheran M, et al. Voltage-gated sodium channel expression in rat spiral ganglion neurons. *Mol Cell Neurosci.* 2009; 42:399–407. [PubMed: 19765660]
112. Hossain WA, Antic SD, Yang Y, et al. Where is the spike generator of the cochlear nerve? Voltage-gated sodium channels in the mouse cochlea. *J Neurosci.* 2005; 25:6857–6868. [PubMed: 16033895]
113. Bakondi G, Por A, Kovacs I, et al. Voltage-gated K⁺ channel (Kv) subunit expression of the guinea pig spiral ganglion cells studied in a newly developed cochlear free-floating preparation. *Brain Res.* 2008; 1210:148–162. [PubMed: 18410910]
114. Hafidi A, Beurg M, Dulon D. Localization and developmental expression of BK channels in mammalian cochlear hair cells. *Neuroscience.* 2005; 130:475–484. [PubMed: 15664704]
115. Yi E, Roux I, Glowatzki E. Dendritic HCN channels shape excitatory postsynaptic potentials at the inner hair cell afferent synapse in the mammalian cochlea. *J Neurophysiol.* 2010; 103:2532–2543. [PubMed: 20220080]
116. Bakondi G, Por A, Kovacs I, et al. Hyperpolarization-activated, cyclic nucleotide-gated, cation non-selective channel subunit expression pattern of guinea-pig spiral ganglion cells. *Neuroscience.* 2009; 158:1469–1477. [PubMed: 19038312]
117. Hisashi K, Nakagawa T, Yasuda T, et al. Voltage-dependent Ca²⁺ channels in the spiral ganglion cells of guinea pig cochlea. *Hear Res.* 1995; 91:196–201. [PubMed: 8647721]
118. Lopez I, Ishiyama G, Acuna D, et al. Immunolocalization of voltage-gated calcium channel alpha1 subunits in the chinchilla cochlea. *Cell Tissue Res.* 2003; 313:177–186. [PubMed: 12845523]
119. Xie D, Hu P, Xiao Z, et al. Subunits of voltage-gated calcium channels in murine spiral ganglion cells. *Acta Otolaryngol.* 2007; 127:8–12. [PubMed: 17364322]
120. Tong M, Hernandez JL, Purcell EK, et al. The intrinsic electrophysiological properties of neurons derived from mouse embryonic stem cells overexpressing neurogenin-1. *Am J Physiol Cell Physiol.* 2010; 299:1335–1344.
121. Marrs GS, Spirou GA. Embryonic assembly of auditory circuits: spiral ganglion and brainstem. *J Physiol.* 2012; 590:2391–2408. [PubMed: 22371481] An excellent demonstration of the changes in electrical activity of auditory neurons during embryonic development
122. Madden C, Hilbert L, Rutter M, et al. Pediatric cochlear implantation in auditory neuropathy. *Otol Neurotol.* 2002; 23:163–168. [PubMed: 11875345]
123. Mason JC, De Michele A, Stevens C, et al. Cochlear implantation in patients with auditory neuropathy of varied etiologies. *Laryngoscope.* 2003; 113:45–49. [PubMed: 12514381]
124. Thorne M, Salt AN, DeMott JE, et al. Cochlear fluid space dimensions for six species derived from reconstructions of three-dimensional magnetic resonance images. *Laryngoscope.* 1999; 109:1661–1668. [PubMed: 10522939]
125. Marzorati S, Pileggi A, Ricordi C. Allogeneic islet transplantation. *Expert Opin Biol Ther.* 2007; 7:1627–1645. [PubMed: 17961088]
126. Lewitzky M, Yamanaka S. Reprogramming somatic cells towards pluripotency by defined factors. *Curr Opin Biotechnol.* 2007; 18:467–473. [PubMed: 18024106]
127. Fu X, Xu Y. Challenges to the clinical application of pluripotent stem cells: towards genomic and functional stability. *Genome Med.* 2012; 4:55. [PubMed: 22741526] An excellent review of the current challenges surrounding the clinical application of pluripotent stem cells
128. Fu X, Xu Y. Self-renewal and scalability of human embryonic stem cells for human therapy. *Regen Med.* 2011; 6:327–334. [PubMed: 21548738]
129. Wise AK, Hume CR, Flynn BO, et al. Effects of localized neurotrophin gene expression on spiral ganglion neuron resprouting in the deafened cochlea. *Mol Ther.* 2010; 18:1111–1122. [PubMed: 20216530]

130. Richardson RT, Wise AK, Thompson BC, et al. Polypyrrole-coated electrodes for the delivery of charge and neurotrophins to cochlear neurons. *Biomaterials*. 2009; 30:2614–2624. [PubMed: 19178943]
131. Zhou Z, Liu Q, Davis RL. Complex regulation of spiral ganglion neuron firing patterns by neurotrophin-3. *J Neurosci*. 2005; 25:7558–7566. [PubMed: 16107643]
132. Newbold C, Richardson R, Huang CQ, et al. An in vitro model for investigating impedance changes with cell growth and electrical stimulation: implications for cochlear implants. *J Neural Eng*. 2004; 1:218–227. [PubMed: 15876642]
133. Millard RE, Shepherd RK. A fully implantable stimulator for use in small laboratory animals. *J Neurosci Methods*. 2007; 166:168–177. [PubMed: 17897719]
134. Lippe WR. Rhythmic spontaneous activity in the developing avian auditory system. *J Neurosci*. 1994; 14:1486–1495. [PubMed: 8126550]
135. Tritsch NX, Yi E, Gale JE, et al. The origin of spontaneous activity in the developing auditory system. *Nature*. 2007; 450:50–55. [PubMed: 17972875]
136. Hansen MR, Zha XM, Bok J, et al. Multiple distinct signal pathways, including an autocrine neurotrophic mechanism, contribute to the survival-promoting effect of depolarization on spiral ganglion neurons in vitro. *J Neurosci*. 2001; 21:2256–2267. [PubMed: 11264301] Describes how electrical stimulation can enhance survival of auditory neurons *in vitro*.
137. Hegarty JL, Kay AR, Green SH. Trophic support of cultured spiral ganglion neurons by depolarization exceeds and is additive with that by neurotrophins or cAMP and requires elevation of [Ca²⁺]_i within a set range. *J Neurosci*. 1997; 17:1959–1970. [PubMed: 9045725]
138. Shepherd RK, Coco A, Epp SB, et al. Chronic depolarization enhances the trophic effects of brain-derived neurotrophic factor in rescuing auditory neurons following a sensorineural hearing loss. *J Comp Neurol*. 2005; 486:145–158. [PubMed: 15844207]
139. Yamada M, Tanemura K, Okada S, et al. Electrical stimulation modulates fate determination of differentiating embryonic stem cells. *Stem Cells*. 2007; 25:562–570. [PubMed: 17110622]
140. Ryugo DK, Kretzmer EA, Niparko JK. Restoration of auditory nerve synapses in cats by cochlear implants. *Science*. 2005; 310:1490–1492. [PubMed: 16322457] Demonstrates that electrical stimulation from a cochlear implant can restore synapses in the central auditory pathway
141. Evans AJ, Thompson BC, Wallace GG, et al. Promoting neurite outgrowth from spiral ganglion neuron explants using polypyrrole/BDNF-coated electrodes. *J Biomed Mater Res A*. 2009; 91:241–250. [PubMed: 18814235]
142. Singh B, Xu QG, Franz CK, et al. Accelerated axon outgrowth, guidance, and target reinnervation across nerve transection gaps following a brief electrical stimulation paradigm. *J Neurosurg*. 2012; 116:498–512. [PubMed: 22149377]
143. Kyoto A, Hata K, Yamashita T. Synapse formation of the cortico-spinal axons is enhanced by RGMa inhibition after spinal cord injury. *Brain Res*. 2007; 1186:74–86. [PubMed: 17996222]
144. Hata K, Fujitani M, Yasuda Y, et al. RGMa inhibition promotes axonal growth and recovery after spinal cord injury. *J Cell Biol*. 2006; 173:47–58. [PubMed: 16585268] Describes the inhibition of repulsive guidance molecules to facilitate process outgrowth and functional recovery in the central nervous system
145. Jongkamonwiwat N, Zine A, Rivolta MN. Stem cell based therapy in the inner ear: appropriate donor cell types and routes for transplantation. *Curr Drug Targets*. 2010; 11:888–897. [PubMed: 20412046]
146. Hyakumura, T.; Dottori, M.; Needham, K., et al. Innervation of peripheral and central auditory tissues by human embryonic stem cell-derived neurons *in vitro* (T-2294). Proceedings of the International Society for Stem Cell Research 10th Annual Meeting; Yokohama, Japan. 2012.
147. Rajala K, Pekkanen-Mattila M, Aalto-Setälä K. Cardiac differentiation of pluripotent stem cells. *Stem Cells Int*. 2011; 2011 383709.
148. Ma J, Guo L, Fiene SJ, et al. High purity human-induced pluripotent stem cell-derived cardiomyocytes: electrophysiological properties of action potentials and ionic currents. *Am J Physiol Heart Circ Physiol*. 2011; 301:H2006–H2017. [PubMed: 21890694]
149. Chiu LL, Iyer RK, Reis LA, et al. Cardiac tissue engineering: current state and perspectives. *Front Biosci*. 2012; 17:1533–1550. [PubMed: 22201819]

150. Singh MS, MacLaren RE. Stem cells as a therapeutic tool for the blind: biology and future prospects. *Proc Biol Sci.* 2011; 278:3009–3016. [PubMed: 21813553]
151. Merabet LB. Building the bionic eye: an emerging reality and opportunity. *Prog Brain Res.* 2011; 192:3–15. [PubMed: 21763515]

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Figure 1.

Histological images illustrate the basic anatomy of the normal hearing mammalian (rat) cochlea. A) Transverse section through the mid-modiolar region of the cochlea, illustrating the fluid-filled turns surrounding the central modiolus and entry of the auditory nerve. B) Higher magnification image of a single turn (inset from A), illustrating in more detail the three fluid-filled compartments: scala vestibuli (SV), scala media (SM) and scala tympani (ST). Note that the cochlear implant is inserted directly into the ST to provide electrical stimulation to the auditory neurons, the cell bodies of which reside within Rosenthal's canal (RC; circled). These neurons extend a peripheral process toward the hair cells (arrows), which are located within the organ of Corti (boxed region), and a central process toward the brainstem. The central processes coalesce to make-up the auditory nerve.

Figure 2.

A comparison of neural activity in primary auditory neurons and human embryonic stem cell-derived neurons. Two examples each of cultured primary auditory neurons (A–B) and stem cell-derived neurons (C–D) displaying a rapidly-adapting firing pattern in response to sustained membrane depolarisation. Firing patterns observed in response to pulse trains (20–200Hz) demonstrate differences in the ability of each neuron to follow higher rates of stimulation (asterisks denote stimulus artefact). E) Action potentials fired by auditory neurons (grey) have shorter latency and are briefer than those displayed by stem cell-derived neurons (black). Voltage-clamp recordings in stem cell-derived neurons display sustained

outward potassium currents evoked by membrane depolarisation (F–G) and fast inward tetrodotoxin-sensitive sodium current (H).

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Table 1

Transplantation strategies for AN replacement

Delivery approach: Scala tympani							Reference
Cell type/preparation	Cell survival time (weeks)	Dispersal	Neural proteins expressed	Glial proteins expressed	Tissue response	AN density	
Guinea pig fetal DRG explant	3	Near ANs	-	-	Little - none (qualitative)	-	[32]
Mouse DRG neuron explants	10	Near medial wall; RC	✓	-	-	-	[31]
Mouse fetal DRG explant, mESC suspension	4	ST, SV, RC	✓	✓	-	-	[37]
Suspension of mouse adult NSCs	4	Near ANs; ST, SV	✓	✓	-	-	[38]
Mouse embryonic DRG neuron explants	6	Mod, near ANs, OoC, ST	✓	-	-	-	[39]
Suspension of partially differentiated mESCs	4	ST, RC	✓	-	Little - none (qualitative)	-	[30]
Suspension of mouse marrow-derived SCs	1	ST, SV, SM	✓	-	-	-	[40]
Suspension of mouse bone-marrow-derived SCs	1	ST, SV, SM	-	-	Little - none (qualitative)	-	[41]
Mouse fetal clonal NSC suspension	6	RC, OoC, ST	✓	✓	-	Deaf +NSCs > Deaf – NSCs	[42]
Suspension of mESCs	2	ST, SV, RC near ANs, Mod near ANFs	✓	✓	-	-	[43]
Suspension of mESCs	4	ST, SV, perilymph of vestibular system	✓	✗	-	-	[44]
Suspension of mESCs	4	ST, Mod, SV, RC, OoC, SL	✓	✓	-	No significant differences	[45]
Suspension of rat embryonic NSCs	2	ST, SV, RC	-	-	-	-	[46]
Mouse embryonic DRG neuron explant	4	ST, RC, near Mod, near ANs	✓	-	-	-	[47]
Suspension of mouse iPSCs	1	ST, Mod	✓	✓	-	-	[48]
Suspension of mouse epithelial SCs	4	SL, RM, ST	-	-	-	-	[49]

Delivery approach: Scala tympani							Reference
Cell type/preparation	Cell survival time (weeks)	Dispersal	Neural proteins expressed	Glial proteins expressed	Tissue response	AN density	
Suspension of human olfactory mucosa-derived SCs	2	ST, SV, SM	-	-	-	-	[50]
Human bone marrow derived MSCs	6	RC	✓	-	Little - none (qualitative)	Deaf+MSCs > Deaf - MSCs	[51]
Suspension of mouse iPSCs	4	ST, SV, SM	✓	-	Teratoma detected in 1/20 cochleae	-	[52]
Delivery approach: Modiolus							Reference
Cell type/preparation	Cell survival time (weeks)	Dispersal	Neuronal proteins expressed	Glial proteins expressed	Tissue response	AN density	
Explant of mouse DRG neurons; suspension of mESCs	9	AN, BS	✓	✓	-	-	[53]
Suspension of chinchilla bone marrow stromal cell	3	SV, ST, SL, RC, Stria, Mod, AN, SM	✓	✓	-	-	[54]
Suspension of mouse fetal NSCs	2	Mod, ST, RC	✓	✓	-	-	[55]
Suspension of mouse embryonic NSCs	4	Mod, IAM, perilymph	✓	✓	-	-	[33]
Suspension of mouse marrow-derived SCs	1	Mod, ST, SV, SM	✓	-	-	-	[40]
Suspension of mouse bone-marrow-derived SCs	1	Mod, ST, SV, SM	-	-	Little - none (qualitative)	-	[41]
Suspension of guinea pig bone marrow stromal cells	4	ST, Mod, IAM	✓	-	-	No significant differences	[56]
Delivery approach: Auditory nerve							Reference
Cell type/preparation	Cell survival time (weeks)	Dispersal	Neuronal proteins expressed	Glial proteins expressed	Tissue response	AN density	
Mouse fetal DRG explant, suspensions of mESCs or mouse adult NSCs	4	BS	✓	-	Little - none (qualitative)	-	[34]

Delivery approach: Scala tympani						
Cell type/preparation	Cell survival time (weeks)	Dispersal	Neural proteins expressed	Glial proteins expressed	Tissue response	AN density
Suspension of mESC-derived neural progenitor cells	14	AN, RC, Mod	-	-	-	-
Suspension of mESCs	5	AN, RC, SM	✓	-	Little - none (qualitative)	-
Suspension of embryonic mouse auditory neuroblasts	9	AN	✓	✗	-	-
Suspension of hESCs	8.6	AN	✓	-	-	-
Delivery approach: Posterior semi-circular canal						
Cell type/preparation	Cell survival time (weeks)	Dispersal	Neuronal proteins expressed	Glial proteins expressed	Tissue response	AN density
Suspension of mESCs	4	SM, ST, RC, Stria	-	-	Little - none (qualitative)	-
Delivery approach: Utricle						
Cell type/preparation	Cell survival time (weeks)	Dispersal	Neuronal proteins expressed	Glial proteins expressed	Tissue response	AN density
Suspension of mESCs	0.6	SV, ST	-	-	-	-
Delivery approach: Rosenthal's canal						
Cell type/preparation	Cell survival time (weeks)	Dispersal	Neuronal proteins expressed	Glial proteins expressed	Tissue response	AN density
Suspension of mESCs	4	RC	✓	✓	-	-
Mouse ESCs encapsulated in hydrogel	1	RC	✓	-	Areolar fibrous tissue – localised to surgical site	Reduced at surgical site, but otherwise unaffected
Delivery approach: Scala media						
Cell type/preparation	Cell survival time (weeks)	Dispersal	Neuronal proteins expressed	Glial proteins expressed	Tissue response	AN density
Suspension of mESCs	4	RC	✓	✓	-	-
Mouse ESCs encapsulated in hydrogel	1	RC	✓	-	Areolar fibrous tissue – localised to surgical site	Reduced at surgical site, but otherwise unaffected

Delivery approach: Scala tympani						
Cell type/preparation	Cell survival time (weeks)	Dispersal	Neural proteins expressed	Glial proteins expressed	Tissue response	AN density
Suspension of mESCs	4	SM	-	-	-	-
Delivery approach: Cochlea lateral wall						
Cell type/preparation	Cell survival time (weeks)	Dispersal	Neuronal proteins expressed	Glial proteins expressed	Tissue response	AN density
Suspension of mouse NSCs	2	SM, SV, ST	-	-	-	-
Delivery approach: Lateral semi-circular canal						
Cell type/preparation	Cell survival time (weeks)	Dispersal	Neuronal proteins expressed	Glial proteins expressed	Tissue response	AN density
Suspension of mouse NSCs	2	SM, SV, ST	-	-	-	-
Delivery approach: Intravenous infusion						
Cell type/preparation	Cell survival time (weeks)	Dispersal	Neuronal proteins expressed	Glial proteins expressed	Tissue response	AN density
Suspension of human cord blood hematopoietic SCs	8.5	ST, SV, bone lining the Mod	✓	-	-	-

DRG – dorsal root ganglion; **SC** – stem cell; **ESC** – embryonic stem cell; **hESC** – human embryonic stem cell; **mESC** – mouse embryonic stem cell; **MSC** – mesenchymal stem cell; **NSC** – neural stem cell; **IPSC** – induced pluripotent stem cell; **ST** – scala tympani; **SV** – scala vestibuli; **SM** – scala media; **Mod** – modiolus; **RC** – Rosenthal's canal; **AN** – AN; **ANF** – auditory nerve fibre; **OoC** – organ of Corti; **SL** – spiral ligament; **BS** – brainstem; **IAM** – internal auditory meatus; **Stria** – stria vascularis;

Table 2

Summary of voltage-dependent currents and contributing ion channel subunits identified in mammalian primary ANs

Currents	Features	Electrophysiology studies	Contributing channel subunits
Voltage-gated sodium currents (I_{Na})	Activation of this current is essential for the rapid depolarisation that underlies the action potential	Santos-Sacchi 1993 [104] (Guinea-pig, adult); Moore et al 1996 [110] (Rat, P6–8); Lin et al 1997 [101] (Gerbil, P0–1)	<u>Tetrodotoxin-sensitive:</u> Nav1.1 (Fryatt et al 2009 [111] [Rat, adult]), Nav1.6 (Fryatt et al 2009 [111] [Rat, adult]; Hossain et al 2005 [112] [Mouse, adult]), Nav1.7 (Fryatt et al 2009 [111] [Rat, adult])
Low-voltage activated potassium currents (I_{KL})	Slowly-inactivating current	Szabo et al 2002 [105] (Guinea pig, adult)	<u>Dendrotoxin-sensitive:</u> Kv1.1 (Adamson et al 2002 [98] [Mouse, P3–8 & adult]; Bakondi et al 2008 [113] [Guinea pig, adult]) Kv1.2 (Bakondi et al 2008 [113] [Guinea pig, adult]) Kv1.6 (Bakondi et al 2008 [113] [Guinea pig, adult])
High-voltage activated potassium currents (I_{KH})		Szabo et al 2002 [105] (Guinea pig, adult)	Kv3.1 (Adamson et al 2002 [98] [Mouse, P3–8 & adult]; Bakondi et al 2008 [113] [Guinea pig, adult])
A-type potassium current (I_A)	Transient, fast-inactivating current	Jagger & Housley 2002 [100] (Rat, P2–6)	Kv1.4 (Bakondi et al 2008 [113] [Guinea pig, adult]) Kv3.4 (Bakondi et al 2008 [113] [Guinea pig, adult]) Kv4.2 (Adamson et al 2002 [98] [Mouse, P3–8 & adult]; Bakondi et al 2008 [113] [Guinea pig, adult]) Kv4.3 (Bakondi et al 2008 [113] [Guinea pig, adult])
Calcium-activated potassium current (I_{KCa})			<u>Large-conductance (BK):</u> BK_{Ca} (Adamson et al 2002 [98] [Mouse, P3–8 & adult]; Hafidi et al 2005 [114] [Mouse, P0–15 & adult])
Hyperpolarisation-activated current (I_h)	Mixed-cation current activated during membrane hyperpolarisation	Chen 1997 [99] (Guinea pig, adult); Mo & Davis 1997 [103] (Mouse, neonatal); Szabo et al 2002 [105] (Guinea pig, adult); Yi et al 2010 [115] (Rat, P7–14)	<u>Hyperpolarisation-activated cyclic nucleotide-gated (HCN) channels:</u> HCN1 (Bakondi et al 2009 [116] [Guinea pig, adult]; Yi et al 2010 [115] [Rat, P9–21]) HCN2 (Bakondi et al 2009 [116] [Guinea pig, adult]; Yi et al 2010 [115] [Rat, P20–21]) HCN3 (Bakondi et al 2009 [116] [Guinea pig, adult]) HCN4 (Bakondi et al 2009 [116] [Guinea pig, adult]; Yi et al 2010 [115] [Rat, P9–21])
High-voltage activated calcium (I_{CaH})		Hisashi et al 1995 [117] (Guinea-pig); Lin et al 1997 [101] (Gerbil, P0–1); Chen et al 2011 [109] (Mouse)	<u>L-type:</u> Ca_v1.2 (Lopez et al 2003 [118] [Chinchilla, adult]; Chen et al 2011 [109] [Mouse, P6–7]) Ca_v1.3 (Lopez et al 2003 [118] [Chinchilla, adult]; Xie et al 2007 [119] [Mouse, P1–3]; Chen et al 2011 [109] [Mouse, P6–7]) <u>P/Q-type:</u> Ca_v2.1 (Chen et al 2011 [109] [Mouse, P6–7]) <u>N-type:</u>

Currents	Features	Electrophysiology studies	Contributing channel subunits
			Ca_v2.2 (Lopez et al 2003 [118] [Chinchilla, adult]; Chen et al 2011 [109] [Mouse, P6–7]) <u>R-type:</u> Ca_v2.3 (Lopez et al 2003 [118] [Chinchilla, adult]; Xie et al 2007 [119] [Mouse, P1–3]; Chen et al 2011 [109] [Mouse, P6–7])
Low-voltage activated calcium (I_{CaL})			<u>T-type:</u> Ca_v3.1 (Chen et al 2011 [109] [Mouse, P6–7]) Ca_v3.3 (Chen et al 2011 [109] [Mouse, P6–7])

Na_V – Voltage-gated sodium channel; K_V – Voltage-gated potassium channel; I_{Na} – Sodium current; I_{KL} – Low-voltage activated potassium current; I_{KH} – High-voltage activated potassium current; I_A – A-type potassium current; I_{KCa} – Calcium-activated potassium current; BK_{Ca} – Large conductance calcium-dependent potassium channel; I_h – Hyperpolarisation-activated current; HCN – Hyperpolarisation-activated cyclic nucleotide-gated channel; I_{CaH} – High-voltage activated calcium current; I_{CaL} – Low-voltage activated calcium current; Ca_V – Voltage-gated calcium channel