A better understanding of hearing loss

3/22/13

For optimal hearing, hair cells located in the inner ear and the spiral ganglion neurons must be correctly connected to each other, since they are the first auditory nerve relays to the central nervous system. If they're not correctly connected, the transduction of sound to the brain is affected, and worse, if no connection is made between these two types of cells, there is no hearing at all. But there are two types of hair cells and spiral ganglion neurons and they cannot come together at random: the inner hair cells connect to type I neurons while the outer hair cells connect to type II neurons. A team of researchers at the University of Liège's GIGA-Neurosciences research unit, led by Brigitte Malgrange, has established that outer hair cells emit a message that repels type I neuron projections to ensure they reach their targets, that is the inner hair cells! This major discovery, which could contribute to hearing restoration, was published in *Nature Communications*.

For most us, hearing is as natural as breathing. Unconsciously, each second of our lives, sounds all around us are sent to the brain and give us information about our environment, allowing us to react, to communicate, or even to relax. As simple and natural as hearing may seem, it can be faulty. According to statistics from the FFSB (French-Speaking Federation of the Deaf in Belgium), around 400,000 people in Belgium are deaf or hearing-impaired, and 40,000 of them are profoundly deaf. In Europe there are 15 million deaf and hearing-impaired people. ONE (Office of Birth and Childhood) estimates that profound hearing loss is detected in one in 1,000 newborns during their first year, and that an additional one in 1,000 newborns are diagnosed during their second year.

At the University of Liège's GIGA-neurosciences research unit, led by Brigitte Malgrange, researchers are trying to understand the development of the auditory portion of the internal ear. "We specifically want to understand why the inner ear cells don't regenerate after having been destroyed," says the FNRS Research Director. (see article "The mysteries of the broken ear").
How sounds travel through the central nervous system

After entering through the auricle and passing the ossicles in the middle ear, sound is transmitted to the auditory portion of the inner ear. "This part is called the **Organ of Corti** and contains the **hair cells** among other things. The stereocilia on the surface of these cells vibrate when sound reaches them. This allows potassium and calcium to enter the hair cells and this modification creates a nerve signal," explains Brigitte Malgrange. This signal is then transmitted to the **neurons** of the **spiral ganglion**, also located in the inner ear. "These neurons are essential because they form the first auditory nerve relay to the central nervous system," states Brigitte Malgrange. For optimal hearing, the hair cells and the spiral ganglion neurons must be correctly connected to one another. If they’re not, the transduction of sound to the brain is affected, and worse, if there is no connection between these two types of cells, there is no hearing at all.
The hair cells and the spiral ganglion neurons don't regenerate after they are destroyed. Cochlear implants, which are the only means we currently have to restore partial hearing in some cases of hearing loss, only work if at least some of the connections between cells are present," indicates the scientist. These very specific connections are made during embryonic development. But there are two types of hair cells and spiral ganglion neurons and they cannot come together at random. The inner hair cells connect to type I neurons while the outer hair cells connect to type II neurons. "The inner hair cells are those that transmit sound while their outer counterparts serve more as frequency adapters," explains Brigitte Malgrange. The most important connection for auditory function is therefore the one between inner hair cells and type I spiral ganglion neurons.

**When the hair cell-neuron pairs are formed**

Jean Defourny has focused on these connections as part of his PhD thesis in the Developmental Neurobiology research unit. "The two types of spiral ganglion neurons emit projections that extend towards the specific hair cells that they must connect to," adds Brigitte Malgrange. "We are interested in the proteins that allow each type of neuron to find the right hair cell."

In the mid-90s, a Swedish research team identified proteins secreted by the hair cells that attracted neuron projections. "It's what we call chemoattraction. Back then the scientists thought that each type of
hair cell produced a different neurotrophin. But during the 2000s, we realized that it wasn’t so simple because we could replace one neurotrophin with another without affecting the neuron-hair cell connections at all," continues Brigitte Malgrange.

In light of this information the researchers from Liège thought that there might also be molecules designed to repel neuron projections. For in order to reach the inner hair cells, the neurites have to pass through the outer hair cells. "Our theory was that the outer hair cells might be sending some kind of message that would repel the type I neuron projections and ensure they reach their targets," explains Brigitte Malgrange.

**Repulsion for better connection**

To explore their theory of chemorepulsion, Jean Defourny and Brigitte Malgrange decided to examine the expression pattern for two types of proteins: Ephrins and their receptors (Ephs). "There are about twenty Ephrins and half as many Eph receptors. We focused on proteins that had a complementary expression pattern in the inner ear. That is, the ones which express Ephrin in the outer hair cells and have receptors in the type I spiral ganglion neurons," states Brigitte Malgrange. That’s how they realized that Ephrin-A5 is exclusively expressed in outer hair cells while its cognate EphA4 receptor is located specifically in the type I neurons. "When the Ephrin-A5 binds to the EphA4 receptor, this creates a repulsion of the type I neuron projections," says the researcher. These results, published in the journal *Nature Communications* (1), were the first to demonstrate the chemorepulsion of neurites by Ephrins in the inner ear.

The researchers tested their initial hypothesis through a series of experiments and in particular through the use of mice which had both proteins removed. "One group of mice didn’t express Ephrin-A5 and the other group didn’t have any EphA4. In both cases our hypothesis proved to be accurate," said Brigitte Malgrange.
A new avenue for hearing restoration

While this discovery is still in the fundamental research stage, in the long term it could lead to therapies to restore hearing in people affected by hearing loss. "If we understand how auditory function develops, we can try to restore connections between the hair cells and the spiral ganglion neurons. Thanks to our research, we now know how it's possible to prevent type I neurons from connecting to outer hair cells," explains the director of the GIGA-neurosciences research unit.

In the near future, Brigitte Malgrange and her team would like to better understand what happens in the actual hair cell-neuron connections. "Here we have shown on a rough scale that when we remove the Ephrin A5 or EphA4 proteins, mice have significant hearing problems. Now we would like to analyse what happens directly in the inner hair cell in regards to neurotransmission," says the scientist. During neurotransmission, the hair cells release a neurotransmitter, glutamate, which binds to the receptors located on the neuron projections. "We would like to see if the absence of Ephrin-A5 or Eph4 directly affects this neurotransmission," states Brigitte Malgrange. Furthermore, she would also like to investigate whether other molecules known to have repulsion properties could also play a role in the establishment of hair cell-spiral ganglion neuron connections.