Title: **Cellular and molecular bases of neuroplasticity: brainstem effects after cochlear damage**

Author: **P. Gil-Loyzaga[1,*], F. Carricondo[1,2], M. V. Bartolomé[1], M. C. Iglesias[3], Rodríguez F[3], Poch-Broto J[3]**.

[1] Laboratory of Neurobiology of Hearing. & Department of Ophthalmology and Otorhinolaryngology. Faculty of Medicine. Complutense University, Madrid, Spain

[2] Biomedical Research Foundation of the "Hospital Clínico San Carlos", Madrid, Spain.

[3] ENT Service, "Hospital Clínico San Carlos", & Department of Ophthalmology and Otorhinolaryngology. Faculty of Medicine. Complutense University, Madrid, Spain.

Running Head: Neuroplasticity in the auditory brainstem

***Address for correspondence:**

Prof. Pablo Gil-Loyzaga, MD PhD.

Universidad Complutense

Apartado de Correos 60.075.

28080 Madrid, Spain

e-mail: loyzaga@med.ucm.es

Abstract

Neuroplasticity is the ability that perform neurons to modify their activity (short or long-term potentiation / depression) and/or their respective synaptic tree organization. Neuroplasticity is a relevant process that leads the animal adaptation to new environments and new situations by the processes of learning and memory. For the human being neuroplasticity has been essential because in clearly involved in the language learning and comprehension. In language tasks neuroplasticity leads the acquisition of a second, third, etc. languages. Neuroplasticity could be important during the application of some therapeutic strategies, in particular in hearing aids or in cochlear implants. After a cochlear lesion or auditory nerve damage afferent connections from auditory ganglion can be highly altered. This situation could result in a clear reduction of auditory input and, of course, an alteration of connectivity of terminals on cochlear nuclei neurons. Such a process could stimulate the reorganization of the neural circuits and neuroplasticity. Cochlea removal has demonstrated to be a good model to analyze brainstem neuroplasticity, in particular when attends to the cochlear nuclei. After cochlea removal three main periods of degeneration and regeneration were observed. Early effects, during the first week after lesion, involved the acute degeneration with nerve ending oedema and degeneration. During the second and, probably, the third weeks after lesion degeneration was still present, even though a limited and diffuse expression of GAP-43 started. Around one month after peripheral lesion degeneration at the cochlear nuclei progressively disappeared and a relevant GAP-43 expression was found.

Key words: neuroplasticity, cochlea, brainstem, cochlear nuclei, auditory system.

Neuroplasticity and sensory systems

Current life provides constantly a very important amount of information to living animals, which is relevant for animal survival. Any environment stimulus, including those from other living species, contains many complementary aspects (f. i. image, sound-noise, odour, temperature, etc.) than serve to define it. But, one sensory system is unable to analyze all of these aspects because each one only perceives one kind of energy. Thus, to really understand the nature of a stimulus the information of all sensory systems must be integrated (1). Therefore, during learning process the neural circuits must be organized to correctly analyze the corresponding kind of information. But also neural circuits must to integrate the information activating one sensory pathway with the rest of the sensory systems. This particular neural activity has been called cross-modal interactions (1,2) and is possible by neuroplasticity processes. Also memory processes, highly relevant during learning and sensory recognition, are only possible by neuroplasticity. Up to now, neurobiologists accepts that neural circuits, using neuroplasticity, are dynamic and adaptable to new situations (2,3,4). In fact, nervous system, even in more advanced species, is only able for correct and adequate neural processing by modelling and remodelling their structure and physiology (4).

Neuroplasticity has been defined as the ability of neurons and circuits to modify: 1) their functional activity (short or long-term potentiation / depression) and/or 2) their synaptic organization in accordance to variations of the activity (1,5-7). Neuroplasticity is present along the individual life span: development, adulthood, after injury, during memory and/or learning, etc. Some neurodegenerative processes show a highly relevant reduction of neuroplasticity from the beginning of degeneration.

Even though neuroplasticity must be especially intense and a key process during development is still present and necessary in the adulthood (4,7). In fact, neuroplasticity is more needed in animals with: 1) a complex psychological activity (in particular mammalians, and especially big apes and humans) and 2) with a long lifespan. Both characteristics together need the preservation of mechanisms that lead a continuous learning and some adaptation processes (4). These processes might generally result in an increase of the use of previous neural circuits, but sometimes also might result in the shape reorganization of synaptic tree (4). But also the maintenance of neuroplasticity activities is necessary for nerve recovery after damage (8).

Neuroplasticity and the auditory pathway

Hearing is a very relevant sensorial activity for human beings because it is necessary during oral language, a relevant psycho-physiological activity (4,9). The auditory pathway exhibits

complex neuroplasticity processes linked to learning language tasks in relationship to increases of functional activity (10). Thus, language is based in learning and memory processes and, its acquisition, results in the organization of neural circuits. But language acquisition is not only restricted to auditory areas, in contrast involves many other cortical regions by cross-modal sensory interactions (4). Therefore, language acquisition and maintenance requires that neuroplasticity, in the auditory and other pathway, remain active along lifespan. All of this is especially relevant during the learning of foreign languages. In fact, learning of "foreign languages" requires a complex reorganization and neuroplasticity (4).

After neural damage neural circuits use the genetically established cross-modal interactions among sensory systems to reorganize the processing of sensory perception. These effects have bee largely confirmed in studies of early sensory deprivation (f. i. congenital blindness or deafness, etc.) (2). Also after auditory damage (e.g. noise trauma, neurotoxicity, etc.) neuroplasticity could result of a correct or an inadequate nerve (or synapses) regeneration (11-14). The use of cochlear implants, micro-pumps or other therapies for deafness requires a review of our knowledge on neuroplasticity.

Substances involved in neuroplasticity.

One of the main goals of the current neurobiology is the searching of chemical factors involved in neuroplasticity. Even though such a process is probably due to the cooperative action of a lot of substances (certainly acting as a molecular cascade as in development of nervous system) a significant amount of recent studies were devoted to define the action of some particular molecules. Experimental studies reveal some important genetic and epigenetic factors involved in neuroplasticity and the different periods when they could act. Some of these factors, currently called neurotrophic factors (15,16), are involved in the organization-reorganization of neural circuits, the guidance of nerve fibres, but also in nerve survival and neurite outgrowth on neurons, in cell culture and after neural damage. Main substances involved in neuroplasticity and neurite outgrowth are: 1) neurotrophic factors (NGF, CNTF, BDNF) acting on cell-membrane tyrosine-kinase receptors (TrkA, TrkB or TrkC) (4,15,17-19); 2) extracellular matrix molecules (f.i. laminin, fibronectin, and other); 3) Cell-adhesion molecules like NCAM, NgCAM, LCAM or calcium-dependent molecules as cadherins, etc. (20); 4) neurotransmitters and other neuroactive substances (f. i. glutamate, GABA, acetyl-choline, serotonin, etc) (5,21-23).; 5) ions; 6), hormones, etc. (see reviews in 3,4,7,24,25). Other molecules as semaphorins or netrins are also relevant for the axon guidance during

neurofasciculation, and after nerve section. The mechanism for triggering off the neuroplasticity remains still quite unknown.

Neuroplasticity and synaptic tree organization and reorganization.

The first synaptic organization and circuits of the sensory systems is genetically-determined and established during embryo life. Thus, the cortical laminar organization of these areas can be observed during these developing periods, but the columnar organization among cortex needs of functional activity (3,26). This clearly indicates that columnar structure will be defined by individual experience and behaviour as in response to environmental stimulation (3,26). Thus, the final synapses tree organization of cortical columnae will be dependent of learning, memory (27).

The arrangement of the synaptic tree is based in the neuron ability to generate cytoplasm elongations called "growth cones", which exhibit continuous protrusion and regression movements (28). This motility is due to the presence of a highly developed cytoskeleton in the growth cones that includes contractile-proteins (actin, myosin and other) (28). Even though the better known are the growth cones linked to axon growing it must be stated that also dendrites elongates using these structures, for instance during neural development (29).

Experimental models of cochlea removal or auditory nerve section.

Experimental models of nerve lesion or sensory deprivation have been considered highly useful to analyze the reorganization and neuroplasticity of neural circuits (2). Several models of auditory nerve section, partial or whole cochlear removal have been used (see review in 8). Animal models (rats or other) submitted to experimental cochlea removal offered a very good model to analyze neuroplasticity in the auditory pathway (30-32). These experiments served to analyze the effects of peripheral auditory deprivation on the cochlear nuclei, as an indication of the effects of deafness.

Several technical possibilities have been developed even though the model more frequently used is the unilateral cochlea removal (8), but partial removal also provided relevant information (33). The effects of cochlea removal on cochlear nuclei have been classically analyzed after short (a week), mid (two weeks) or chronic periods (more than a month after removal) (8).

The effects of cochlea deprivation and regeneration have been evidenced by the study of neural protein distribution within the cochlear nuclei. Two interesting neural proteins have been used: the synaptophysin (Syp) which is a highly functional protein in mature synapses and the

growth association protein (GAP-43) which appears in developing and regenerating nerve-ending buttons.

The synaptophysin (Syp) is a nerve ending protein of 38 kDa that has been largely found in synapses of neurons (f. i. in: brain, spinal cord, retina, neuromuscular junctions, adrenal cells etc.) when reached the maturity (34-41). It has been clarified that Syp participates in neurotransmission during the fusion of presynaptic vesicles to the presynaptic membrane and the release of neurotransmitters (36-40,42-44).

The Syp expression appeared in the majority (probably all) the synapses that can be found within the CN (8). It could be also used as a complementary method to identify and classify the CN synaptic nerve endings by their shape and size, following the classical types: large cup-shaped buttons, round-ovoid buttons and small buttons. But the most relevant finding is that Syp expression exhibits a conspicuous distribution within the CN in mammals (45,46). In fact, big differences between the ventral cochlear nucleus (VCN) and the dorsal cochlear nucleus (DCN) were found (8). A regular pattern of large-mid size buttons were found in the VCN surrounding the neuron cells bodies and nerve fibres, while a dense and homogeneous network of very small puncta shaped buttons appeared at the DCN (45). Also, neurons of the cochlear nerve root area, between AVCN and PVCN, appeared surrounded by large nerve endings containing Syp (8). In contrast, the neuropil between neurons was largely free of synaptic Syp-containing buttons except for projections of nerve fibres (8).

Differences of size and shape of synaptic buttons, identified by Syp expression, may be related to the type of neuron activity. The anatomical regions of the CN exhibit a different function (47). While VCN is mainly involved in the reception of primary afferents (tonotopically organized), while the DCN could be mainly involved in a first analysis of the information that reach it from the VCN.

The GAP-43 is a calmodulin binding phosphoprotein currently found in growing axons and growth cones of developing neurons (48-56), but also in regenerating axons (48,53,57,58). This protein is down-regulated when nerve fibres start connection to their corresponding targets. The expression of GAP-43 might indicate the existence of a regeneration or neuroplasticity process, such as long term potentiation (30,53,58). GAP-43 is considered a useful marker of developmental neuron connections (55) and neuroplasticity or regenerating nerve fibres (53). The Syp expression seems to be an efficient marker to identify mature synapses and GAP-43 expression serves to recognize new buttons or regenerating fibres (53,56). But a complete evaluation CN changes after cochlea removal needs also the use of transmission electron microscopy.

Cochlear nuclei changes after cochlea removal or after auditory nerve section.

The cochlear nuclei (CN) complex has a conspicuous anatomical division in three main parts. Each part contains distinct cell types and a particular nerve fibre distribution (59-61). The three main subnuclei are: anterior ventral (AVCN), posterior ventral (PVCN) and dorsal (DCN) cochlear nuclei.

In our experimental model control animals exhibited a normal structure and neuronal and neuropil distribution. Thus a dense amount of healthy round and oval neuronal cell bodies can be observed at the antero-ventral cochlear nucleus (AVCN) (Fig. 1 a), but it was similar for the postero-ventral cochlear nucleus (PVCN). This completely normal structure can be found in non operated cochlear nuclei from both the control animals and the contro-lateral nucleus to the operated side. Also, synaptic distribution in control animals was highly regular including big primary afferent buttons (Fig. 1 b, arrows) and other puncta and small buttons.

In a previous publication (8) three main cochlear nuclei degeneration phases after cochlea removal were proposed:

1- Phase of acute degeneration of the CN

A very short time (from hours to 1 day) after cochlea removal the most immediate morphological findings, in particular the nerve fibre oedema, were observed (Fig. 1c). These initial degeneration effects, with abundant rounded-shape afferent nerve fibers (Fig. 1c), were identified by using transmission electron microscopy, because it lets to reveal minimal ultrastructural changes. However at this time CN neuron cell bodies appeared apparently healthy. However, neuron cell death was also found in CN after cochlea removal in particular in susceptible animals (31,62). These findings matched well with other previous studies that found CN acute degeneration started immediately after cochlear removal or auditory deprivation (31,63). Also a precocious and significant reduction of Syp (synaptophysin) expression has been described in rats (8) and in guinea pigs after cochlea removal (46). This effect will be present in long-term survival animals (see the third experimental group of animals). No relevant degeneration effect or ultrastructural changes were observed in the DCN. Preliminary signs of neuron plasticity were noticed by the analysis of the expression of a developmental and transient neural protein (GAP-43 expression), because this molecule has been frequently used as a clear indicator of regenerating axons (48).

2- Phase of stabilization of lesions at the CN and start of neuroplasticity

The second period started around a week after lesion and is characterized by a progressive increase of neuroplasticity. During this second degeneration period a rapid neuron cell death was observed (Fig. 1 d). This finding matched well with the fact that, 2 months after deafening,

a dramatic reduction of neurons was noticed. It seems that only less the 25% of neuron population was still present at that time, when compared to the deafened and non-deafened animals (32). A significant change in the distribution of Syp expression was observed in both parts of the VCN, in fact the big projections, end-bulbs of Held, have disappeared, but some small buttons were found surrounding the VCN neurons (8).

In fact, a relevant increase of new nerve fibres containing GAP-43 was detected along the VCN. Neuron cell bodies were found completely surrounded by small nerve endings and punctae containing GAP-34 (Figure. 1 e). This abundant small nerve endings might correspond to projections from the commissural fibres from no-deprived side (64) and/or from the descending auditory pathway (65,66).

3- Phase of neuroplasticity and CN reorganization that takes place around after two weeks and a month after cochlea removal.

At this last phase after cochlear removal a certain number of neurons still appeared with signs of degeneration (8). But the more relevant finding of this period was the predominance of the regeneration process. It is clear that the CN morphology remains deeply modify by the cochlea removal (Fig. 2).

Significant differences were observed when compared the Syp expression at the CN complex between animals submitted to cochlear ablation and controls two weeks or more after lesion. While no-deafened animals exhibited an abundant Syp expression in the ventral cochlear nuclei (Fig. 2a), animals that were submitted to cochlear removal (model in Fig. 2b) showed a high reduction of Syp expression in the same VCN (Fig. 2 c). Also, a relevant increase of GAP-43 expression was reported in the deafened CN complex (8).

Relevance of neuroplasticity in CN complex after cochlea removal

The neuroplasticity in the mammalian nervous system is linked to the activity of several molecules as growth factors (67,68), neurotransmitters (68,69), among other. In a our studies after auditory receptor lesion or deafening some relevant changes were found in the nerve fibre organization and distribution of nerve fibres by using two complementary markers: Syp, as an indicator of functional synapses, and GAP-43 which recognized growing nerve fibres.

The experimental partial or whole cochlear removal in animals was chosen as a good model of auditory peripheral lesion or deafening with neuronal degeneration (31,70). A first interesting finding is that very quickly after cochlea removal CN neurons rapidly degenerate (31), which could be linked to several complementary situations: oxidative stress, excitotoxicity and a significant auditory activity reduction (8,71,72). Certainly, a very interesting finding was to

recognize that in the next days after cochlea removal or nerve section started neuroplasticity. Thin nerve fibres and terminals were found growing and replacing the big afferent auditory terminals previously degenerated. Trophic factors must be responsible of such a regenerative process. Also, glutamate and NMDA receptors have been involved in neo-fasciculation in both in vivo (73) and in vitro experiments (74). Neuroplasticity is still an intriguing process with many factor and sequences of activities involved. Neuroplasticity has been claimed usually as a positive process because might help to regenerate nerve and fascicles in the peripheral but also in the central auditory system. However, a bad connectivity after neuroplasticity processes could be also responsible of several pathologies including dislexia among other (60).

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Legends of figures.

Fig. 1. a) Ultrastructure (Transmission Electron-Microscopy) of a panoramic view of the ventral cochlear nucleus. Oval and round-shape neurons (stars) are observed in this image with a healthy and no deleterious effects appear.
b- Syp expression in the ventral cochlear nucleus of a control no-deafened rat. Positive dark reactive points (arrows) correspond to typical thick end-bulbs of primary afferent nerve fibres making contact with CN neurons.
c) Ultrastructure (Transmission Electron-Microscopy) of the ventral cochlear nucleus, of a rat 1 day after cochlea removal, showing oedematous afferent nerve fibres (arrows).
d) Ultrastructure (Transmission Electron-Microscopy) semi-panoramic view of the ventral cochlear nucleus, of a rat 5 days after cochlea removal, showing some neuron degeneration image (arrows).
e) GAP expression in the ventral cochlear nuclei, of a rat 3 days after cochlear removal, demonstrated by dark small points around neuron cell bodies (arrows).

Fig. 2. a) Syp expression in the cochlear nuclei complex of a no-deafened rat (panoramic view). Syp expression has a unlike distribution in either ventral (VCN) and dorsal (DCN) cochlear nuclei. In the VCN, Syp expression exhibits a more intense presence in large nerve endings around neurons. In contrast, in the DCN exhibits a dense regular pattern.
b) Schematic drawn showing of the cochlea and cochlear (CN) complex before nerve section or cochlea removal (scalpel) following the dot discontinuous line
c) Cochlear nuclei (CN) effects 15 days after cochlea removal. Syp expression is highly reduced in both the AVCN and PVCN, while seems to be unaffected in the DCN, except in its deep region.

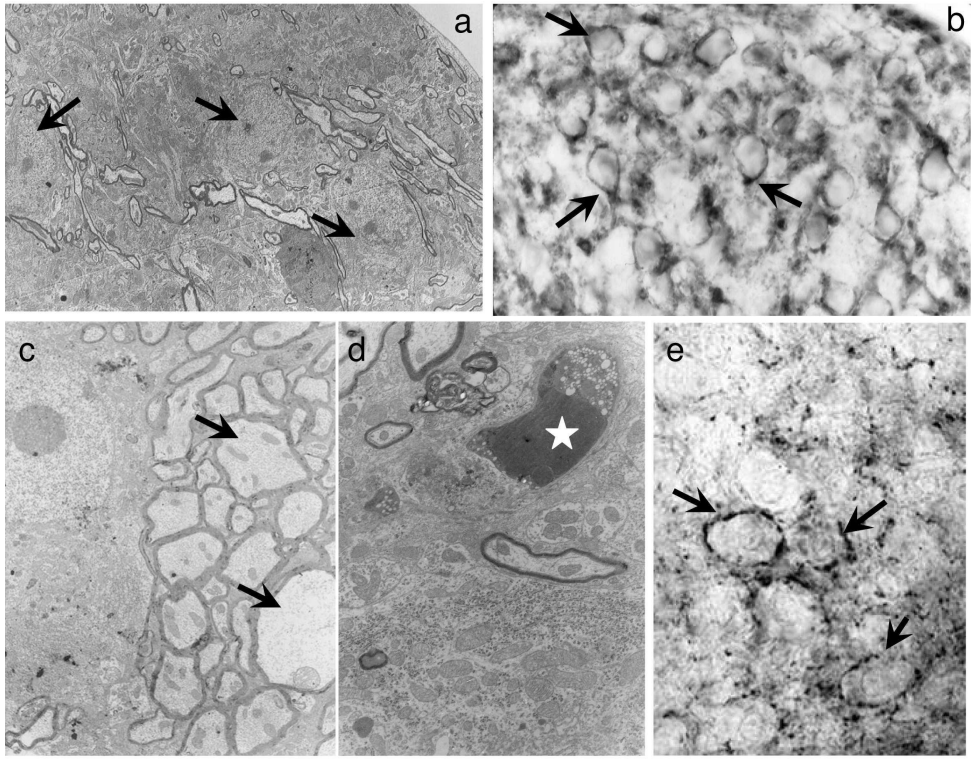

Figure 1
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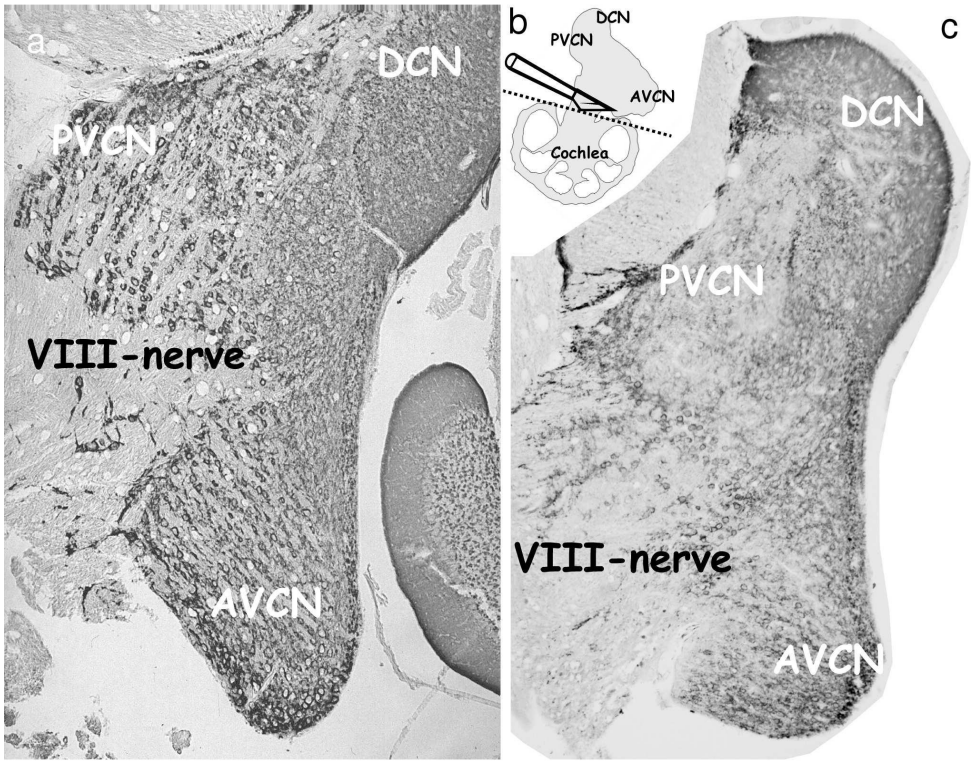

Figure 2
171x136mm (300 x 300 DPI)