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Cellular regulatory mechanisms influencing the activity of the cochlear nucleus: a review

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The cochlear nucleus is the site in the auditory pathway where the primary sensory information carried by the fibres of the acoustic nerve is transmitted to the second-order neurones. According to the generally accepted view this transmission is not a simple relay process but is considered as the first stage where the decoding of the auditory information begins. This notion is based on the diverse neurone composition and highly ordered structure of the nucleus, on the complex electrophysiological properties and activity patterns of the neurones, on the activity of local and descending modulatory mechanisms and on the presence of a highly sophisticated intracellular Ca^{2+} homeostasis. This review puts emphasis on introducing the experimental findings supporting the above statements and on the questions which should be answered in order to gain a better understanding of the function of the cochlear nucleus.

Keywords: auditory function, cochlear nucleus, neuronal electrophysiology, regulation of cytoplasmic Ca^{2+}

Hearing is a sensory function that emerged in the course of the phylogenesis as responding to pressure waves originating in the environment. Invertebrate and low level vertebrate aquatic animals, for example, are able to sense the streams of the surrounding water and to use this information for coordinating their movements and for orientation in space. Sound waves propagating in the air are similarly important in the life of terrestrial species. Besides signaling the environment, however, sound perception (and generation) gradually became a fundamental tool for communication as well.

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Outline of auditory information processing

The increasing importance of hearing makes it quite obvious that the coding/decoding task of the auditory pathway had to grow more and more complicated. The acoustic information is eventually embedded into the physical parameters of a bunch of airwaves (e.g. frequency and energy content). These quantities are first transformed by the hair cells of the cochlea, partly by the tonotopic arrangement of the receptors (responsible primarily for coding the pitch and tone), partly by the number of the responding cells and the size of the generated receptor potential (encoding mainly the intensity). The propagating neuronal information (action potential activity) is born in the primary sensory neurones that are also the first structure to carry the information into the higher auditory centers. During the receptor potential/action potential transformation both the tonotopic order and the quantitative features of excitation should be maintained to make the further steps of neuronal decoding as faithful as possible. Moreover, an additional parameter, namely the temporal relationship of the action potential trains generated bilaterally is evaluated by comparing them. This latter task can be fulfilled only if the timing of the activity patterns arriving from the individual cochleae is also precisely preserved.

Abbreviations used in this paper:

4-AP, 4-aminopyridine
AMPA, α-amino-3-hydroxy-5-methyl-isoxazole proprionic acid
AVCN, anteroventral cochlear nucleus
$[Ca^{2+}]_i$, cytoplasmic Ca^{2+} concentration
CICR, Ca^{2+} induced Ca^{2+} release
CN, cochlear nucleus
CNS, central nervous system
DCN, dorsal cochlear nucleus
EPSC, excitatory postsynaptic current
EPSP, excitatory postsynaptic potential
GABA, gamma-aminobutyric acid
HVA, high-voltage activated
IICR, inositol 1,4,5-trisphosphate induced Ca^{2+} release
IP_3, inositol 1,4,5-trisphosphate
IPSP, inhibitory postsynaptic potential
KA, kainic acid
LVA, low-voltage activated
MNTB, medial nucleus of the trapezoid body
NM, magnocellular nucleus
NMDA, N-methyl-D-aspartate
PMCA, plasmalemmal Ca^{2+} (pump)
PVCN, posteroventral cochlear nucleus
SERCA, sarco-endoplasmic reticular Ca^{2+} (pump)
SOC, superior olivary complex
t-ACPD, (±)-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid
TEA^+, tetraethylammonium ion
UBC, unipolar brush cell
VCN, ventral cochlear nucleus

In mammals the primary sensory fibres, i.e. the fibres of the acoustic (or 8th cranial) nerve carry the action potentials into the cochlear nucleus (CN) complex where the second-order sensory neurones are located. The nucleus can be divided into ventral (VCN) and dorsal (DCN) parts, each made of several neurone types. Both the VCN and the DCN receive primary auditory information and both of them send projections to third-order centers (superior olivary complex, SOC; medial nucleus of the trapezoid body, MNTB). The second-order axons originating from the CN partly remain ipsilateral, but some of them cross the midline and project to contralaterally situated areas. This arrangement makes the third-order neurones able to compare the bilaterally generated action potential trains.

In order to elucidate the cellular background of the hearing function, several experiments were carried out on different avian species. In these animals a neurone population called magnocellular nucleus (NM) functions as second-order sensory center, consequently, it is considered as the avian analogue of the CN. The NM neurones project to third-order sensory nuclei (e.g. to the lateral nucleus).

The present review primarily intends to give a summary of the available information about the neuronal processes determining the activity of the second-order auditory nuclei (CN and NM, respectively). There is a growing body of evidence that these structures fulfill more complex function than being just a relay station, as the first steps of the decoding seem to take place in these nuclei. It is also known that the CN is the target of descending pathways that modulate the signal processing of the various intrinsic neuronal networks. Such a complex neuronal activity has to involve several transmembrane and intracellular signal transduction pathways. Our attention will be focused on the structural and electrophysiological aspects of the information transfer in CN and on those regulatory functions in which the cytoplasmic $[Ca^{2+}]_i$ is either effector or target. In those cases, however, where only limited information is available regarding CN and NM neurones, we incorporated data from other cell types in order to indicate some open questions and possible answers.

General structure of the CN complex

The processing of the auditory information in the CN begins with a certain degree of analysis of the action potential pattern arriving via the auditory nerve. As a result, the various components of the auditory stimuli are passed onto different pathways, which often run in parallel with each other, and feed information to the subsequent centres of the auditory system. It is known that some of these pathways can maintain the temporal coding of the acoustic signals, while in other ones temporal coding and high fidelity transmission do not seem to be important.

In both the ventral and dorsal parts of the CN there is a considerable degree of organisation in the arrangement of the different cell types. In the ventral region of the CN two clearly different areas can be determined. One of these is the multipolar cell area, which contains mostly stellate and some bushy neurones (155). This region is located very close to the root of the acoustic nerve. The other region of VCN is the octopus cell area, situated in the caudal region of the nucleus, where the auditory nerve fibres are very closely bundled.

The DCN in mammals seems to be even more significantly organised than the ventral region, as in this area one can define four concentrically arranged layers (11). The most superficial area is the molecular layer (layer 1), containing mainly the axons of the granule cells, although some granule cell bodies are also situated here. Immediately below the molecular layer the fusiform (or the pyramidal) layer is found (layer 2), where the cell bodies of the pyramidal, granule, cartwheel and stellate cells are situated. In layer 3 the basal dendritic tree of the pyramidal cells is found, along with some vertical cells. The descending fibres of the acoustic nerve also terminate here and contact these structures. The deepest part of the nucleus (layer 4) consists of mainly the cell bodies of the giant and large multipolar cells.

Obviously, the most important source of information to the CN is the acoustic nerve itself (81), but this nucleus receives acetylcholinergic modulatory projections as well from the SOC. There are two types of cochlear nerve fibres making contact with the CN (25, 100). Thick, myelinated type I fibres convey information from the inner hair cells of the organ of Corti but the CN is also contacted by type II fibres which are much thinner, possess no myelin sheath and originate from the outer hair cells of the inner ear. While type I fibres contact some of the biggest cells of the CN complex (i.e. pyramidal and bushy neurones), type II nerve fibres reach the granule cells, which neurones distribute the received information via the extensive parallel fibre network.

As a significant degree of signal processing takes place in the CN, it is important to realize that the major structures targeted by the efferent pathways of the CN are the SOC, the inferior colliculus, the ventral nucleus of the lateral lemniscus and the MNTB.

The most important cell types of the CN, their projections and major characteristics

Bushy cells

Bushy cells have a rather characteristic appearance, with rounded or oval cell body from which a thick primary dendrite is originating, giving rise to a complex dendritic tree. The “bushy” term (11) reflects the typical look of these neurones that are situated in the VCN only. Two subtypes of these cells have been described (44, 45).

Spherical bushy cells have cell bodies whose diameter is in the 20–28 μm range. The dendritic tree is complicated and the numerous branches of the dendrites are usually situated in the proximity of the cell body. Spherical bushy cells are often situated in rows adjacent to the acoustic nerve fibres, and they often form clusters consisting of 4–6 individual cells whose dendritic trees show considerable overlapping. Interestingly, although the dendritic tree of these cells is rather complex, providing a huge area on which synaptic connections could be formed, they are relatively free of synaptic inputs. In fact, the spherical bushy cells receive information directly from the acoustic nerve via giant synapses (endbulbs of Held, (73)), making contact with the cell body. Some secondary synapses also reach the proximal dendritic trunks, but they leave the apical dendrites free of synaptic inputs. The distal segments of the dendrites are covered by an astrocyte sheath, and they might be involved in producing cell-to-cell interactions with each other (16). The axons of the spherical bushy cells leave the CN and provide input to the ipsilateral medial and lateral olivary nuclei. These cells produce "primary-like" activity pattern, as they are capable of maintaining the temporal features of the action potential pattern provided by the acoustic nerve.

The other major type of the bushy cells is the globular bushy cells. Their bodies are roughly the same as the somata of the spherical cells, but their dendritic tree is more elongated, and the processes are somewhat courser and sparser. As the result of the more elongated and less dense dendritic arborization, globular cell somata are not so much surrounded by these processes as in the case of the spherical bushy cells. Globular cells are usually situated in the root of the acoustic nerve, and they show no obvious clustering. Both the ascending and descending cochlear nerve fibres contact their cell bodies. These synapses are somewhat smaller than the endbulbs, but several times bigger than conventional boutons. Altogether some 85% of the cell body is involved in forming the synaptic connections with the acoustic nerve (141), and similarly to spherical cells, much of the dendritic tree is free of synapses (139, 141), posing the same intriguing question about the possible function of a complex, although synapse-free dendritic arborization. Globular bushy cells also produce "primary-like" responses (111, 155) which are conducted to the contralateral MNTB, where the axons of the globular bushy cells form another giant synapse (calyx of Held) on the cell bodies of the principal cells.

Electrophysiological investigations conducted on enzymatically isolated neurones of the guinea pig CN (75) revealed that the cell type which most likely corresponded to the bushy neurones produced very rapidly adapting (or type II) response pattern, where only a few action potentials could be observed at the initiation of a depolarizing stimulus. A low-threshold outward current was found, with fairly slow activation that seemed to be partly activated at the resting membrane potential. Putative bushy cells also possessed a high-threshold current that could be activated at membrane potentials

positive to –35 mV. The former current component was sensitive to 4-aminopyridine (4-AP) and only moderately affected by tetraethylammonium ions (TEA^+), while the latter type of current was sensitive to TEA^+ but insensitive to 4-AP. It was postulated that the low-threshold component had an essential contribution to preserving the temporal coding of the auditory information originating from the organ of Corti.

Similar conclusion was reached in experiments on gerbil (126). It was shown that the inhibition of either K^+ conductance (and the resulting depolarization tendency) converted the rapidly adapting firing of the bushy cells to a sustained activity. The presence of a persistent, although TTX sensitive Na^+ conductance was also reported whose activation seemed to contribute to the depolarization forces affecting these neurones.

The unique synaptic apparatus of the bushy neurones allowed the investigators to directly access the giant sized presynaptic terminals of these neurones in the MNTB. As the investigation of the membrane currents of both their presynaptic terminals and cell bodies are possible a unique opportunity is provided to directly compare the membrane characteristics and ionic conductances at these locations. So far the Ca^{2+} currents and the hyperpolarization-activated non-specific cationic conductance have been contrasted (21, 24). It turned out that although both the cell body and the presynaptic terminal possess depolarization-activated Ca^{2+} conductances, the two sets of channels are not identical. While the presynaptic terminal did not seem to express T-type Ca^{2+} channels, it could be identified on the cell body. Moreover, on the cell body the P/Q type Ca^{2+} current was responsible for the genesis of only some 6% of the total Ca^{2+} current, in the presynaptic terminal this was the only type of Ca^{2+} current which seemed to be present. Although no evidence for the activation of the remaining types of Ca^{2+} channels (N-, R- and L-types) could be observed on the presynaptic terminal, these subpopulations were identified on the somata, and they had approximately equal contribution to the total Ca^{2+} current recorded. It has also been shown that somatic high-voltage activated (HVA) Ca^{2+} currents could be reduced by the activation of metabotropic glutamatergic receptors on the cell bodies.

The comparison of the hyperpolarization-activated currents of the two locations revealed only smaller differences. Long-lasting hyperpolarizations caused the activation of a slowly activating and non-inactivating inward current, which could be identified as a non-specific cationic current (I_h). Although the major properties of this current (steady-state activation, activation time course, reversal potential) were very similar at both locations, an interesting difference in their Ba^{2+} sensitivities was found. The somatic current could be inhibited to a certain extent by applying 1 mmol/l $BaCl_2$ in the extracellular solution, while such blocking effect was not seen in the case of the presynaptic terminal. However, the current showed strong Cs^+ sensitivity at both locations.

Octopus cells

Just like bushy cells, octopus cells are also situated in the VCN (45). These are relatively big cells (the diameter of the cell body is some 30–35 μm) with rounded body. Their characteristic appearance is provided by the several thick dendrites which tend to protrude from only one particular side of the body, resembling the shape of an octopus (96). Octopus cells receive information from the acoustic nerve. Their dendrites cross the descending branches of the acoustic nerve at the point where the cochlear fibres show the most significant degree of convergence, allowing the octopus cells to make contact with the highest possible number of these axons. On stimulation they produce an "onset" firing pattern (thus they fire action potential only at the beginning of the stimulation), hence they are capable of maintaining information concerning the time of onset of the auditory stimulus (111, 146).

In fact, octopus cells seem to be capable of detecting the coincidence of simultaneous firing of a population of acoustic nerve fibres, and they pass the information about this coincidence with high temporal precision. Specific investigations also revealed that octopus cells can produce this characteristic, really high fidelity transmission, as a result of the combined presence and activity of a hyperpolarization-activated mixed cationic current (I_h) and a low-threshold K^+ conductance (4, 40). Quite surprisingly the half-activation voltage of the h-current identified on octopus cells was some –65 mV, in contrast to the E_{50} values reported in the cases of bushy cell bodies and presynaptic terminals which were approximately 30 mV more negative. This relatively positive half-activation voltage observed in the octopus cells makes possible that 41% of the total h-conductance is activated at the resting membrane potential (about –60 mV), providing therefore a significant inward current which can be observed at rest. Removal of cAMP and cGMP from the pipette solution caused the activation of this current to shift towards more negative membrane potentials.

As the consequence of these ionic conductances, octopus cells have extraordinarily low input resistance (2.4 MΩ) and very low membrane time constants (0.21 ms). These characteristics of the membrane mean that octopus cells require large depolarizing currents for the initiation of the action potentials, and the result is the genesis of a singularly appearing, relatively low amplitude, very brief (0.24–0.54 ms) tetrodotoxin-sensitive action potential.

The axons of the octopus cells form the intermediate acoustic stria, projecting to the periolivary cell groups of the SOC. It is noteworthy that the thickest axonal outputs of the CN are formed by the bushy cells and the octopus neurones, giving the capability of high fidelity and high velocity transmission, ensuring very precise time coding of the acoustic information.

Stellate cells

Stellate cells of the VCN do not form a homogeneous cell population, but possibly are composed of several different cell subtypes with rather different size (the largest neurones have cell body diameters up to 35 μm; while the small cells are only 15–25 μm in diameter) (15, 44). These neurones are primarily situated anterior and posterior to the nerve root, and they possess rounded or somewhat elongated cell bodies. The dendritic tree radiates away from the somata, and consists of mainly long, straight processes which do not branch significantly. These medium diameter processes expand significantly and in the cases of the bigger cells cover a 400 μm region around the cell, while in the smaller cells they are rarely longer than 200 μm. Stellate cells receive synaptic inputs from the auditory nerve fibre collaterals which make contact with the cell body and the dendrites as well. Stellate cells seem to get information from a broad band of auditory fibres. They produce a chopper response to auditory input (111), and (at least some of these neurones) project to the inferior colliculus (the chopper firing pattern can be described as the genesis of a train of the action potentials followed by a short silent period, then a train of action potentials again and so on). It is noteworthy that the axons of the stellate cells are much thinner than those of the bushy and octopus neurones projecting to the SOC, indicating that the timing of the information provided by the stellate cells is not so vital for the processing of the auditory information.

There are indications that stellate cells are spontaneously active, and their action potentials are always followed by large and brief afterhyperpolarizations (2). When sustained depolarization is applied, stellate cells produce a series of action potentials (type I firing pattern) which is in clear contrast with the rapidly adapting type II behaviour of the bushy cells. It was demonstrated (75) that cells in a freshly isolated preparation which could be classified as stellate cells possessed a non-inactivating outward current. This current component could be activated by depolarizations positive to –35 mV and showed TEA^+ sensitivity. On the basis of these characteristics and considering the fact that its reversal potential was reasonably close to the calculated Nernst potential of K^+, it was concluded that this current component was indeed a K^+ conductance.

Some authors (92) differentiated between T- and D-type stellate cells of the posteroventral cochlear nucleus (PVCN) of the mice. T-stellate cells have 0.5–1.1 μm thick axons, and they can be observed in or near to the MNTB. Most of these axons have collaterals in the PVCN too. The terminal regions of their dendrites have highly branched endings, situated near to the auditory fibres. Shocks applied to the root of the acoustic nerve caused them to produce a short latency excitatory postsynaptic potential (EPSP) and a longer latency inhibitory postsynaptic potential (IPSP). When a sustained

depolarization was applied they fired regular and not overshooting action potentials, followed by a single undershoot. The input resistance of these cells turned out to be relatively low, about 65–75 MΩ. The function of these cells is still dubious, but it seems certain that they are excitatory neurones and they respond to stimuli as "sustained choppers". They certainly project to the contralateral inferior colliculus, and it has been also suggested that they may eventually project to the ipsilateral inferior colliculus as well.

Much less is known about D-stellate cells, however. It seems that their dendrites are smooth, long and only rarely branching. The axons of these cells terminate in both PVCN and DCN, but their targets have not been described yet. It is noteworthy that D-cells are associated with areas where granule cells have relatively high density, so some kind of interaction between these cell types seems to be likely. D-stellate cells appear to be inhibitory neurones, producing an "onset/chopper" firing pattern in response to a maintained tone.

Fan cells

Fan cells (often referred as elongate cells) are small cells situated in the central region of the DCN (11, 23, 74, 146). The cell bodies have a characteristic oval shape with a dimension of 15×20 μm. These cells have 100–200 μm long, wavy dendrites that tend to branch towards the two poles of the cell. Fan cells found just above the acoustic stria (hence at the bottom of the nucleus) are always oriented horizontally.

The majority of the fan cell dendrites project to the central areas of the DCN, although there are some processes which reach the granule cell layer. However, no dendritic processes have been found in the molecular layer. Interestingly, no evidence has been produced demonstrating direct contact between the fan cells and the acoustic nerve fibres, despite the fact that these dendrites end in the termination area of the acoustic nerve. Fan cells seem to be local interneurones.

Radiate cells

Radiate cells are large, multipolar cells, situated mainly in the deep regions of the DCN. When investigated with light microscopy, they can be easily confused with giant cells, but they can be differentiated on the basis of their Nissl structure (96). It is also worth mentioning that rodent species usually possess only few giant cells. In fact most of the cells identified as giant neurones are radiate cells. These cells have irregular soma with a diameter of 30–35 μm. The dendrites of these cells enter the granule cell layer and terminate at the border with the molecular layer. It seems that radiate cell dendrites are contacted with primary acoustic nerve terminals, and receive information from a

broad band of acoustic nerve fibre (128). In turn these types of cell project to the inferior colliculus, via relatively thin axons, hence the timing of the information they provide may not be essential for the further steps of the processing of the acoustic signals.

Cartwheel cells

Cell bodies of the cartwheel cells are found in the superficial part of the fusiform as well as in the molecular layer of the DCN. Cartwheel cells were first described in the cat (11), but they are also numerous in the rabbit, mouse, rat and guinea pig. In these species a significant number of cartwheel cells can be observed, in fact they are outnumbered by the granule cells only. As the name suggests their dendrites are radiating away from the cell body similarly to the spokes of a wheel. These processes cross the molecular layer and reach the outer margin of the nucleus. This anatomical arrangement allows that one cartwheel cell is contacted by a large number of cochlear parallel fibres, but no primary acoustic terminals have been described making contact with either the cell body or the dendrites of the cartwheel cells (152, 153). Their axons do not leave the CN, but terminate in a rather restricted area of the DCN, which includes the fusiform cell layer as well as the deep region of the nucleus, hence they seem to be interneurones of the CN.

Cartwheel cells have been shown to be glutamate decarboxylase positive, suggesting that they are probably inhibitory (1). It was found (84) that the activity of cartwheel cells could be opposed by the application of 1 μmol/l strychnine but not if 100 μmol/l picrotoxin was employed, indicating that they might be glycinergic neurones. However, it was also reported (38) that not all cartwheel cells showed positive immunoreaction to glycine, indicating that in the cartwheel cell population only one subset is glycine-immunoreactive.

Cartwheel cells seem to be homologous to Purkinje cells of the cerebellum, as they are both participating in local neuronal circuits, and both cells are positive to gamma-aminobutyric acid [(GABA (84)], GAD (1), PEP19 (6) and cerebellin (86).

Cartwheel cells have a resting membrane potential of –50 to –68 mV, their input resistance is 140 MΩ on average (157). It is noteworthy that in another report (2) basically similar membrane properties and behaviour of the cartwheel cells were described, but these results showed somewhat smaller input resistances (~70 MΩ), accompanied with membrane time constants of some 9.6 ms. Cartwheel cells produce spontaneous depolarizations, which result in spontaneous action potential firing. However, it is believed that these slow depolarizations are the consequences of excitatory synaptic inputs originating from the granule neurones of the CN. When activated, cartwheel cells produce a burst of action potentials which is superimposed onto a slower depolarization. In fact, cartwheel cells seem to be the only cell type in the

CN being capable of producing bursts of action potentials. The rapid firing is TTX sensitive, while the slow depolarization is probably associated with activation of Ca^{2+} channels indicated by the fact that 100 μmol/l verapamil completely abolished these slow depolarizations. During the action potential train, the amplitude of the individual action potentials progressively reduces and they are often followed by double undershoots. Application of TEA^+ or 4-AP shifted the resting membrane potentials of these cells to 20 mV more positive values, indicating that a significant proportion of K^+ channels are activated at rest, contributing to the resting membrane characteristics of these neurones.

Fusiform cells

Fusiform (also known as pyramidal) cells are found in the DCN only. They possess characteristic oval or triangular soma with a diameter of some 25 μm (96, 146). They have basal and apical dendritic trees extending from the body at right angles (73, 74, 111, 146). The apical dendritic tree intrudes the molecular layer of the nucleus, forms an approximately conical arborization and makes several contacts with the parallel fibre network there. This dendritic arborization is usually smaller than 400 μm in diameter. The basal dendrites, on the other hand are relatively free of appendages, but have a beady appearance. These processes are contacted with primary acoustic nerve fibres, originating from a relatively restricted area of the cochlea. The acoustic nerve also makes contact with the cell bodies of the pyramidal cells, in both cases large, spherical synapses have been reported (57, 128). The axons enter the dorsal acoustic stria and eventually pass into the contralateral inferior colliculus (97, 123). Like other cell types projecting to the inferior colliculus, pyramidal cell axons are also relatively thin, making it unlikely that they can maintain the temporal coding of the incoming acoustic stimuli. In cats, the axons give rise to collateral terminals making contact within the DCN. In mice, on the other hand, the axons seem to be free of collaterals.

When the acoustic nerve root is stimulated the pyramidal cells produce a relatively short period of intense activity, which is followed by a longer period of inhibition. This behaviour is termed “pauser” or “buildup” firing, and is thought to be the consequence of direct activation provided by the acoustic nerve fibres terminating on mainly the cell bodies. A smaller contribution is made by the inputs terminating on the dendrites of the pyramidal cells. The inhibition, on the other hand, is due to the activation of some inhibitory interneurones, which are indirectly activated by the acoustic nerve fibres, possibly via the parallel fibre network.

The resting membrane potential of the pyramidal cells is usually between –50 and –60 mV, and these neurones possess a relatively low input resistance of some 86 MΩ (160). Fusiform cells regularly produce frequent, spontaneous IPSPs appearing either

alone or in bursts. Some of these IPSPs were preceded by spontaneous excitatory synaptic inputs. It has been suggested that granule cells may be involved in the genesis of these spontaneous activities recorded from the pyramidal neurones.

Pyramidal cells can produce three distinct firing patterns in response to sound, these activities are known as “pauser”, “chopper” and “buildup” firing characteristics. It has been shown (59) that the availability of a rapidly inactivating A-type K^+ current, which was relatively insensitive to the application of TEA^+ had essential roles in determining the type of response pattern observed after stimulation. Besides this rapidly inactivating current, a more slowly inactivating, TEA^+ and 4-AP sensitive component was also described. Moreover, a rapidly activating current was also demonstrated on enzymatically isolated pyramidal neurones. Besides these transient current components, it was described (48) that pyramidal cells possessed a persistent Na^+ current as well, which became activated near the resting membrane potential.

Pyramidal cells also express a huge variety of depolarization-activated Ca^{2+} currents (41). In fact every known type of Ca^{2+} current has been identified in the membrane of the fusiform neurones, and the presence of some of the Ca^{2+} channel components was confirmed by using immunocytochemistry. It also turned out that at –30 mV the N-type Ca^{2+} channels had the most significant contribution to the total Ca^{2+} current, while at depolarizations to 0 mV the contribution of the L-type current became far more significant. It is also noteworthy that activation of $GABA_B$ receptors could reversibly depress the amplitude of the N-type Ca^{2+} current, indicating that this Ca^{2+} channel subtype may be involved in the various neuromodulatory mechanisms which might also affect the firing characteristics of these cells.

It is worth mentioning that in humans the DCN lacks the molecular and granule cell layers, and the laminar cytoarchitecture is also missing (82). Although the pyramidal cells are also missing, the dominant cell type in the human DCN is an elongated cell type that appears to be identical with neurones classified as pyramidal cells in other species.

Giant cells

Giant cells are the biggest cells in the CN complex, they are found in both the ventral and dorsal parts of the nucleus (96). These neurones are truly big, they have cell bodies with some 50 μm in diameter, and their nuclei and nucleoli are similarly oversized. Although in the sagittal section they show unusually large, tri- or multiangular cell bodies, when observed in other planes, the cell body appears to be narrow and elongated, indicating that giant cells in fact are rather flat. They have been described in several species including rodents and cat, although more giant cells have been reported in the cat than in rodent species (44, 45, 82, 146).

It is often complicated to differentiate between the giant cells, and the largest multipolar cells of the VCN or radiate cells of the DCN. It is a helpful hint, however, that giant cells have longer and thicker dendritic processes as well as a more distinctly multiangular cell body than the other two cell types.

The dendritic arborisation of the giant cells covers a huge area in the CN, often extending to 500–600 μm. It seems that this cell type provides the only large caliber dendrites which penetrate the granule cell layer in the VCN and the molecular layer of the DCN. As a result of the large area covered by their dendrites they have a huge receptor field, and can receive information from an extraordinarily large number of acoustic nerve fibres, hence they seem to be capable of integrating inputs from a wide band of acoustic nerve fibres (112). This function is indicated also by the findings of functional studies, showing that they produce an unusually large frequency and dynamic range of action potentials. Giant cells have a rather extensive projection area as well, as their axons and axon collaterals reach the contralateral inferior colliculus and the ipsi- and contralateral CN complex (97, 123).

Giant cells produce large, overshooting action potentials that are followed by two afterhyperpolarizations (158). It has been noted that the first undershoot drives the membrane potential to more negative values than that of the resting membrane potential, regardless of the intensity of the stimulus initiating the firing. The second afterhyperpolarization, on the other hand, was found to be much more variable in its amplitude. On the basis of the information yielded by investigating the responses of the giant cells to the stimulation of either the acoustic nerve root or the AVCN, it seems that giant cells are activated directly by the acoustic nerve fibres, as well as by excitatory interneurones of the VCN (granule and T stellate cells), which are either mono- or polysynaptically coupled to the giant neurones. The tuberculoventral cells, on the other hand, provide glycinergic inhibitory projections to the giant cells which can be neutralised by the application of 1 μmol/l strychnine.

Granule cells

As the giant cells are the largest cells in the whole CN, including both ventral and dorsal parts, the granule cells are the smallest and most numerous cells in the entire CN complex (87). They possess very small rounded cell bodies, which are some 6–8 μm in diameter, hence they are somewhat bigger than their cerebellar counterparts. The dendrites of the cochlear granule cells are also somewhat longer (70 μm) than those of the cerebellum, these processes tend to end in small forks or claws. Granule cells, although they are present in both parts of the CN, tend to be situated rather peripherically, well outside the usual termination zone of the acoustic nerve (28, 74). However, even these regions may be reached by acoustic nerve collaterals; and there are

some granule cell areas in the central region of the nucleus as well, where they may be contacted either via axon collaterals or by the primary endings of the acoustic nerve (57). Granule cells also receive information from the cochlear mossy fibres originating from the olivocochlear bundle, inferior colliculus as well as from the dorsal and ventral nuclei of the lateral lemniscus. These types of neurones are also contacted by fibres originating from the cuneate nuclei. Granule cells are involved in local nuclear circuits, as their axons form the parallel fibre network, which contacts some rather prominent cell types (e.g. pyramidal cells) of the nucleus.

Considering the fact that granule cells receive information from both the acoustic system and the cuneate nuclei, they seem to be important in forming certain neuronal circuits within the nucleus, which may integrate somatosensory (originating for example from the pinna) and acoustic information. Such circuits may be important in controlling the movements of the ears. However, when one takes into account the almost complete lack of pinna movements in primates, it should not be surprising that granule cells and the related machinery is rather insignificant in primates. In fact, in the primate CN the granule, star, cartwheel cells and the molecular layer of the DCN are very much reduced both in number and in size.

Some authors suggested that granule cells might be spontaneously active. This activity may be the result of either their intrinsic membrane properties or may be driven by excitatory inputs converging on granule cells. The spontaneous firing of the granule cells may eventually lead to firing of all neurones contacted by granule cell processes, including the pyramidal cells, some of the stellate cells and cartwheel neurones.

There are only a few data available on the membrane characteristics of the granule cells, as their relatively small size did not make them a particularly attractive target for electrophysiological measurements. However, they seem to fire a train of action potentials on long-lasting depolarizations, and they have very low threshold for action potential firing, as current amplitudes of 10–35 pA could evoke firing of these neurones (117). The extreme sensitivity of the granule cells is most likely to be associated with their exceptionally high input resistance (~2.7 GΩ). The voltage-clamp measurements revealed that granule cells possess at least two types of depolarization activated K^+ currents. An A-type component seems to control the latency of the action potential firing on depolarization, depending on the prior membrane potential of the granule cells and therefore on the availability of this current component. A much slower component, on the other hand, provides the repolarizing power of these cells after the action potentials.

Golgi cells

Golgi cells are not present in the guinea pig CN. In other species, where they are found they are thought to be GABAergic inhibitory interneurones, whose cell bodies are in the molecular and fusiform cell layers and whose axons terminate locally within the CN, mainly in the granule cell domain. Their axonal arborization is particularly striking as it may cover an area up to 200–400 μm in diameter, especially in the proximity of the granule cells, indicating that the targets of these axons are probably granule cells. Compared to the rest of the cell types in the CN complex, Golgi cells have relatively large input resistance (some 130 MΩ), and they are capable of producing regular, overshooting action potentials (29). It is very likely that they receive direct synaptic inputs from the acoustic nerve, as the activation of the cochlear nerve root evoked EPSPs on the Golgi cells with a relatively long latency (1.3 ms). This finding rises the possibility that Golgi cells are contacted by slowly conducting, non-myelinated acoustic fibres. Golgi cells are also stimulated by granule cells, and it seems that this effect is mediated via NMDA receptors.

Golgi cells were found to be the most intensively GABA-positive cells at birth in the granule cell domain (39). Later on, during the postnatal development, the immunoreactivity to GABA and glycine increased in other, previously weakly responding cell types as well. The staining pattern observed in adult animals is reached at about two weeks after the onset of hearing.

It is believed that Golgi cells are capable to integrate multimodal influences as they are parts of neuronal circuits which are also targeted by the granule cells of the CN (29).

Star cells

Although the name of the star cells essentially means stellate, they should be differentiated from the previously discussed, and generally much bigger stellate cells (11, 74, 146, 152). Star cells are situated in the granule and molecular layers of the VCN and DCN, respectively. Besides these locations, star cells are also often found in the granule cell lamina present between the VCN and DCN, and along the acoustic stria, where a peripheral accumulation of the granule cells is located. Star cells have thin, straight dendrites, radiating to some 150–200 μm away from the body, although there are also smaller star cells, where the length of the dendrites is just 100 μm. Generally, most dendrites leave the granule cell layer or granule cell areas, and reach the central regions of the VCN and DCN. The dendrites seem to make synaptic connections with parallel fibres. Although the distal dendrites project into the termination area of the acoustic nerve fibres, no direct evidence has been presented so far to show that they receive direct innervation from the acoustic nerve itself.

Possibly one of the most exciting observations about star cells is that they form dendro-dendritic and dendro-somatic gap-junctions, producing therefore a network of electrically coupled cells. This network engulfs the whole CN, but the exact function of this system is not known.

Tuberculoventral association cells

The cell bodies of the tuberculoventral association cells (occasionally also referred as vertical or corn cells) are found in the deep region of the DCN, while their dendrites are situated in both the deep and the fusiform layers (74). These dendrites are irregularly thick and thin, giving a rather pronounced beaded appearance of these processes. These dendritic projections are situated in relatively narrow (70–100 μm wide) bands situated in parallel with the acoustic nerve fibres. Tuberculoventral cells receive inputs from type I auditory nerve fibres as well as from excitatory interneurones (probably T stellate cells) of the CN. It seems that inhibitory projections provided by the D stellate cells of the VCN also target them. Corn cells, in turn, project to the ipsilateral VCN but their axons also terminate on pyramidal cells (147). The distribution of the tuberculoventral cells is identical to that of glycine positive ones, indicating that they are possibly inhibitory neurones, hence they would be capable of providing frequency-dependent inhibition to the pyramidal neurones and to the VCN (148).

In slices prepared from the mouse DCN, tuberculoventral association cells did not receive spontaneous synaptic inputs in a significant quantity, neither themselves produced spontaneous activity. However, application of depolarizing current stimuli could evoke regular action potential firing. The action potentials usually showed overshooting and were followed by two afterhyperpolarizations, which did not produce undershooting when larger depolarizing currents were applied. The action potential firing and the shape of the action potentials were not affected by the application of verapamil, indicating that Ca^{2+} channel activation had a negligible role if any in the genesis of the firing characteristics of these neurones. 4-AP depolarized the tuberculoventral cells to such an extent that prevented the firing of action potentials, indicating the significance of K^+ channels in determining the resting and active membrane properties of these neurones. TEA^+, on the other hand, completely removed the afterhyperpolarizations that usually followed action potential firing, and increased the duration of the action potentials (159).

Unipolar brush cell

Unipolar brush cells (UBCs) form a class of a novel cell type with relatively small size (85). These neurones are situated both in the cerebellum, mainly in the

granule cell layer of the cerebellar cortex and in the granule cell domains of the DCN. They have rather characteristic structure, as they possess a single dendrite whose ending forms a brush-like structure. In the cerebellum these dendrites are in close and unusually extensive synaptic contact with mossy fibres. It has been suggested that this synaptic arrangement may trap the synaptic transmitters in the synaptic clefts. These neurones possess both ionotropic and metabotropic glutamatergic receptors. On the other hand, some nerve fibres which make contact with the UBCs are positive to choline acetyltransferase, hence it seems rather likely that co-transmission of glutamate and acetylcholine occurs in the synapses reaching the UBCs. The dendrites of these neurones make dendro-dendritic contacts with the granule cells, in which synapses they appear to be excitatory (110).

Calcium-related regulatory mechanisms in the CN and NM neurones

Regulation and role of the cytoplasmic [Ca^{2+}] in neurones

It has been realized a long time ago that several forms of cellular activity are preceded or accompanied by an increase in the cytoplasmic Ca^{2+} concentration $[Ca^{2+}]_i$. The best-known Ca^{2+}-related phenomena include muscular contraction, exocrine and endocrine secretions, mitosis, etc. In neurones the role of Ca^{2+} entry in triggering the release of neurotransmitters was also pointed out several decades ago. Due to the methodological difficulties, however, a comprehensive survey of the Ca^{2+}-related control of neuronal functions was delayed until recent developments, especially the widespread use of fluorescent Ca^{2+} indicators made possible to collect the necessary data. In this section a short overview of the components of the neuronal Ca^{2+} homeostasis is given while for detailed description of the related topics excellent reviews (e.g. 8, 89, 105, 129, 134) and books (e.g. 27, 67) may be consulted.

Calcium ions accumulating in the cytoplasm may originate from the extracellular space. Local depolarizations and action potential firing are able to activate voltage-gated Ca^{2+} channels in the surface membrane. Considering the variable voltage dependencies and kinetic features of these channels (32, 129) one may suppose that they play important roles both in the electrogenesis (low-voltage activated or LVA channels) and in triggering Ca^{2+} activated intracellular processes following depolarizations (high-voltage activated or HVA channels). The chemical synaptic transmission between neurones involves the activity of ionotropic receptors, and many of these ligand-gated channels are permeable to Ca^{2+}. The rather numerous glutamatergic receptors (49) play especially important roles but other ligand gated cationic receptor channels are also present in the CNS and contribute to the Ca^{2+} loading of the postsynaptic neurones.

The other source of the cytoplasmic Ca^{2+} is the assembly of intracellular Ca^{2+} stores that reside in the endoplasmic reticulum (134) and require a primary signal to release the stored Ca^{2+}. Ca^{2+} induced Ca^{2+} release (CICR) is based on the presence of the ryanodine receptor Ca^{2+} channels in the stores (46). Under physiological conditions Ca^{2+} entering from the extracellular space may act as the activator of the CICR mechanism which, in turn, helps the faster build-up of the cytoplasmic $[Ca^{2+}]$ elevation. Similarly to other cell types, opening of the ryanodine recptors in neurones can be achieved by pharmacological agents like caffeine (132). Another population of the neuronal intracellular Ca^{2+} stores is activated by inositol 1,4,5-trisphosphate (IP_3). The production of IP_3 is frequently connected to the activity of metabotropic receptors that can act via G proteins and phospholypase C (3, 134). The IP_3 receptors of the stores also function as Ca^{2+} release channels (IP_3 induced Ca^{2+} release, IICR (134)).

The proper operation of many homeostatic regulatory mechanisms requires that after the development of the desired effect the system returns to its initial (resting) state to be ready to initiate a new activity cycle. This principle is essentially true for the Ca^{2+} dependent mechanisms although in this case the summation of the individual regulatory phases carries additional information (see e.g. the hypotheses about the development of long-term potentiation (20, 22, 36). It is also known, however, that unusually high or long-lasting intracellular Ca^{2+} transients in several cells including neurones may have serious consequences like excitotoxicity, degenerative diseases, etc. (e.g. (78)). It is not surprising, consequently, that all the known mechanisms able to decrease $[Ca^{2+}]_i$ are present in neurones.

Ca^{2+} entering the cytoplasm can be buffered by Ca^{2+} binding proteins. It is a well established correlation that the higher the intracellular Ca^{2+} buffer capacity in a cell, the smaller $[Ca^{2+}]_i$ changes can be produced by the cell in question ((31) and references therein). It was also described that neurones containing considerable amounts of Ca^{2+} binding proteins are more resistant to the excitotoxic effects under pathological conditions than those presenting smaller buffer capacity (143).

Buffering proteins provide an effective but temporary defense mechanism, hence Ca^{2+} must eventually leave the cytoplasm. Intracellular Ca^{2+} stores may serve as a sink due to the powerful SERCA (sarco-endoplasmic reticular Ca^{2+}) pump present in their membrane (134). Ca^{2+} taken up by the stores may be available for further release although some data indicate that the neurones may deplete the Ca^{2+} content of the stores without significant elevations of the $[Ca^{2+}]_i$ (132).

In addition to the specific intracellular storing sites, Ca^{2+} may be accumulated by the mitochondria especially in case of particularly significant Ca^{2+} loading (47). If the $[Ca^{2+}]_i$ exceeds a certain limit, mitochondrial Ca^{2+} uptake is activated, while after lowering $[Ca^{2+}]_i$ the accumulated ions leave the mitochondria again. This mechanism

implicates that mitochondria may function as Ca^{2+} source following previous loading and the Ca^{2+} leaving them may contribute to the pathogenesis of neurotoxicity (90).

On a long run the most straightforward way of decreasing $[Ca^{2+}]_i$ is to transport Ca^{2+} into the extracellular space. This task is fulfilled partly by a primary active pump PMCA (17), partly by the Na^+/Ca^{2+} exchange mechanism (9, 104). Both transport routes are present in the surface membrane of the neurones, however, their contribution to the overall removal of Ca^{2+} from the intracellular space shows considerable neurone to neurone variability.

Depolarization-evoked intracellular Ca^{2+} transients in the CN neurones

The presence of voltage gated Ca^{2+} channels in the surface membrane of the neurones explains that a considerable amount of Ca^{2+} enter the neuronal cytoplasm on depolarizations increasing $[Ca^{2+}]$ here. These depolarization-evoked $[Ca^{2+}]_i$ elevations (transients) have been described in several regions of the CNS although the results show a considerable scattering regarding the quantitative parameters of the transients (size, time course, etc.). Nevertheless, the available data indicate clearly that depolarizations induce increases of the $[Ca^{2+}]_i$ in acutely dissociated neurones, in nerve cells grown in tissue culture and in neurones investigated in slice preparations ((67) and references therein). The depolarizations were elicited by different means, e.g. by generating action potentials, by applying depolarizing pulses under voltage clamp conditions or by elevating external $[K^+]$ (67). Considering this variety of experimental conditions the above-mentioned scattering of the data can be easily understood.

When studying the pharmacological modifications of Ca^{2+} currents in pyramidal neurones of the rat DCN, it was revealed that these cells express all known subtypes of the voltage-gated Ca^{2+} channels (41). Similar conclusion was reached from experiments on non-specified guinea pig DCN neurones (80). Moreover, the presence of P/Q- as well as N-type Ca^{2+} channel $\alpha 1$ subunits was justified also by using immuno-cytochemical methods (19, 41). It was reasonable to suppose, consequently, that these cells could produce Ca^{2+} transients on depolarizations. Performing Fura-2 fluorimetric experiments on acutely isolated pyramidal neurones of the rat DCN it was indeed shown that on elevating the external $[K^+]$ to 10–50 mmol/l, $[Ca^{2+}]_i$ increases developed in a dose-dependent manner (118). The maximum amplitude of these transients was in a range of 250–320 nmol/l (43), close to the values reported earlier on other types of neurones.

Considering the diversity of the voltage-gated Ca^{2+} channels, questions emerge about the relative importance of the individual subtypes in generating the Ca^{2+} transients. The contribution of a given Ca^{2+} channel subtype to the overall Ca^{2+} entry is determined by several factors as:

i) the relative density of the individual subtypes;

ii) the Ca^{2+} conductance values;

iii) the relation of the activation threshold to the actual membrane potential value during depolarization. The question has been addressed on several cell types by using different Ca^{2+} channel blockers and analysing the modifications of the Ca^{2+} transients in response. In part of these studies some divalent cations were applied utilising the information that low concentrations of Cd^{2+} seemed to block mainly the HVA currents while Ni^{2+} preferentially inhibited the LVA (T-type) currents in various neurone populations (32, 50, 129).

The effects of Cd^{2+} and Ni^{2+} on depolarization induced Ca^{2+} transients in various neurones suggested that LVA channels were present mainly in the dendritic membrane while HVA channels were more numerous in the membrane of somata and axonal ending (18, 70). Data from isolated rat DCN pyramidal cells also reflected a greater blocking effect of Cd^{2+} than that of Ni^{2+} on K^+ depolarization induced Ca^{2+} transients (43). As in these cells the isolation process results in an almost complete loss of the dendritic tree (119), this finding is in accordance with the uneven distribution of the LVA and HVA channel subtypes, where the latter ones dominate in the somatic membrane.

One of the fundamental differences between HVA and LVA Ca^{2+} channels is the value of their activation threshold. Even the names of the two groups refer to the fact that T-type channels are activated at more negative membrane potentials (i.e. on smaller depolarizations) while the other subtypes require more positive voltages (stronger depolarizations) for opening. Consequently, on evoking Ca^{2+} transients by applying small depolarizations, the T-type channels provide a more significant contribution to the Ca^{2+} entry, while in the case of stronger depolarizations the share of the various HVA channels becomes much more important. Earlier data from rat cerebellar neurones were perfectly in line with the above prediction (65). Experiments on isolated rat DCN pyramidal cells also indicated that the extent of the K^+ depolarization may have a role in determining the routes involved in the generation of the Ca^{2+} transients (118).

Recent developments made it clear that the HVA Ca^{2+} channel subtypes can be further separated by applying toxin blockers specific to the individual channels. These toxins were widely used for mapping the Ca^{2+} channel composition in the membrane of various neurone types but have been sparsely utilised for testing the role of the HVA channel subtypes in eliciting depolarization induced Ca^{2+} transients (see e.g. (65)). Experiments on freshly dissociated rat DCN pyramidal cells (118) demonstrated that the Ca^{2+} transients could be most effectively blocked by using ω-conotoxin GVIA, a specific blocker of the N-type Ca^{2+} channels, while nifedipine (an L-type channel blocker) and ω-agatoxin IVA (a P/Q-type channel inhibitor) were less effective. This

finding was in accordance with the results of patch-clamp measurements on the same cell type, which also indicated a substantial contribution of the N-type Ca^{2+} channels to the total HVA Ca^{2+} current (41).

Reports describing depolarization-induced Ca^{2+} transients of neurones agree that on repolarization the $[Ca^{2+}]_i$ quickly returns to its resting level. It is also generally accepted that this decay is the consequence of the combined action of several clearance mechanisms. Beyond these concordant statements, however, there is a considerable uncertainty regarding the specific role of the various transport processes in individual neurone types and among the given experimental conditions. Studies aimed at analyzing this question followed two major strategies. Some authors tried to find the best mathematical description of the falling phase of the Ca^{2+} transients and used the rate constants obtained to identify the clearance mechanisms involved. These studies resulted in controversial results as in some cases a single rate constant was enough to describe the overall Ca^{2+} removal process satisfactorily (see e.g. (140, 143)) and references therein) while in other instances a double exponential function was required to achieve the best fit (see e.g. (30)). Moreover, in a few reports even more complicated time course of the decay of the Ca^{2+} transients was shown (77). The pyramidal neurones isolated from the rat DCN themselves reflected the above complexity as the falling phase of their Ca^{2+} transients could be fitted to either one or two exponential functions depending on the size of the preceding Ca^{2+} load (42). Moreover, in the case of the biggest Ca^{2+} transients a plateau-like shape of the falling phase could be observed.

Although the variability of the results can be attributed at least partly to the rather diverse experimental conditions (source of the neurones, isolation methods, etc.), one has to conclude that the identification of the individual transport processes on the basis of their kinetic parameters alone is not a straightforward approach. As the number of the Ca^{2+} removal mechanisms involved is certainly higher than the time constants reported, one may suppose that the individual transport rates are probably close to each other, consequently, they cannot be resolved by the fitting procedures. To overcome this problem, in an alternative line of studies pharmacological interventions known to modify the individual Ca^{2+} transporters were applied and the consequences of these manoeuvres were analysed by calculating again the time constants of the decay phases of the Ca^{2+} transients. Although this strategy seems to be more effective, the experiments revealed that the available pharmacological tools are usually not specific enough to give unambiguous results. Moreover, the transport processes in question proved to be interdependent and were able to compensate for each other's impaired function. These complications make it understandable why the results of the pharmacological modifications provided progress only to a limited extent so far. In general, the involvement of all known Ca^{2+} removal mechanisms could be shown, including the SERCA pump, PMCA pump, Na^+/Ca^{2+} exchange and mitochondria (see

e.g. (30, 77, 140)). However, it has to be emphasized again that these and similar reports have been published several contradictory data, indicating that the relative importance of the individual transport mechanisms may have considerable variability depending on cell types and (possibly) on experimental conditions as well.

In the cases of isolated rat DCN pyramidal cells the pharmacological modifications proved the primary roles of the SERCA pump and the PMCA pump route in decreasing $[Ca^{2+}]_i$ following depolarizations (42). It was also established that blockade of the Na^+/Ca^{2+} exchange mechanism did not cause major change in the kinetics of the Ca^{2+} transients if the other clearance pathways were available. The role of the mitochondria has not been tested in these cells, consequently, definitive conclusions regarding the importance of the individual Ca^{2+} transport mechanisms cannot be drawn. It is noteworthy, however, that in the avian NM the role of the mitochondria in the post-load Ca^{2+} removal seemed to be of primary importance while the participation of the endoplasmic reticulum was less evident (83). Nevertheless, the presence and age-related expression changes of the SERCA pump protein have been shown also in NM neurones (162).

Cytoplasmic Ca^{2+} binding proteins in CN and NM neurones

The three major Ca^{2+} binding proteins that can be widely found in the cytoplasm of the neurones include parvalbumin, calretinin and calbindin. Several attempts have been made to map these molecules in the CNS of various species using different techniques. Some studies applied polyclonal or monoclonal antibodies, while in more recent experiments the presence of the corresponding mRNAs was tested using appropriate methods. In general it can be stated that the occurrence of all three proteins in the central acoustic neurones has been proved. Parvalbumin was found in most of the neurones of the auditory pathway in guinea pig (13, 135) and rat (72, 135) as well as in the VCN octopus cells of the gerbil (66). Calretinin was described in the somata of several CN neurones of rat (72), in various VCN neurone types of the guinea pig (13, 151), in UBCs of the marmoset (136, 137) as well as in chicken NM neurones (99, 138).

As the above list indicates parvalbumin and calretinin are located mainly in the ventral neurones of the CN. On the contrary, calbindin is found mostly in DCN neurones. This Ca^{2+} binding protein is characteristic of the cartwheel cells in guinea pig (13, 135), rat (135) and chinchilla (35). It is also present in the fusiform cells of chinchilla (35), in Purkinje-like cells of rat (51, 135) and guinea pig (135) as well as in the UBCs of the marmoset (137). It seems to be particularly interesting that in the horseshoe bat a generally low or even missing calbindin immunoreactivity was described (144), while in the mustached bat calbindin was found in both VCN and DCN

neurones, only spherical bushy cells (VCN) and pyramidal neurones (DCN) seemed to lack this protein (156).

In some studies attempts were made to compare the localisation of the different Ca^{2+} binding proteins. In guinea pig calretinin and calbindin staining did not show any overlap (13) while in rat true co-localisation of the two proteins in VCN neurones was reported (114). Moreover, indication of a calretinin/calbindin interchangeability was described in the marmoset (137). These findings seem to suggest that the expression of a given Ca^{2+} binding protein may have some implications regarding the specific functions of the given neurone but the number of the available data is far not enough to establish correlation and reach definitive conclusions.

As it was pointed out earlier the presence of the intracellular binding proteins yields a Ca^{2+} buffering capacity protecting the neurones against the cytotoxic effects of high or long-lasting $[Ca^{2+}]_i$ elevations. Estimating the effectivity of this buffer mechanism, however, requires quantitative description of the elements of the Ca^{2+} homeostasis (105). Such data were not available for rat DCN pyramidal neurones, consequently, when their cytoplasmic Ca^{2+} buffering power was discussed, the controversial indirect results could not be reconciled (42).

Although the lack of the parameter values renders difficult to construct a quantitative model of Ca^{2+} handling, some observations allow insight into the possible physiological significance of the neuronal Ca^{2+} buffering proteins. Several reports have shown that the expression level of these proteins changes with age. In rats, for example, parvalbumin appears at about the 8^{th} postnatal day and its expression increases to the adult level while calretinin is already present 2 days before birth, then its amount decreases at some locations along the auditory pathway (72). It was also reported that calbindin appears around the 2^{nd} postnatal day in neurones of the rat central auditory system, its expression increases gradually and around the 24^{th} postnatal day it reaches the adult level (34). Developmental changes of calretinin expression in chicken brainstem auditory neurones were also noted (99). These observations clearly indicate that the presence of Ca^{2+} binding proteins may be important for the synaptic refinement taking place during the development of the hearing function.

Another important clue of understanding the function of Ca^{2+} binding proteins in the auditory pathway is provided by the so-called deafferentation experiments. It is known that the termination of the acoustic nerve input to the CN or NM changes the Ca^{2+} homeostasis of the second-order sensory neurones located here (see later). After the deafferentation in certain CN neurones of guinea pig an early increase of parvalbumin and calretinin expression (12, 151) and a secondary, late decrease of calretinin content were described (151). The transiently increased $[Ca^{2+}]_i$ buffering capacity of the neuronal cytoplasm seems to be a protective mechanism against the elevated $[Ca^{2+}]_i$ often seen following sensory deprivation (see later). According to a

recent hypothesis (163) deafferentation leads to increased $[Ca^{2+}]_i$ in CN neurones (e.g. by activating AMPA receptors) which, in turn, activates gene transcription of Ca^{2+} binding proteins (via the calcium/calmodulin kinase and protein kinase A pathways). It is noteworthy, however, that in chicken NM, where the deafferentation induced $[Ca^{2+}]_i$ increases are also significant, calretinin concentration seems to be unchanged following deafferentation (99, 138). These results indicate a rather complex function of cytoplasmic Ca^{2+} buffers in maintaining Ca^{2+} homeostasis, especially in mammalian species.

This function could also be noted under pathological conditions. In genetically deaf mice the age-dependent deterioration and the consequently decreased activity of the cochlear hair cells and spiral ganglion neurones were accompanied by a moderate increase in the number of parvalbumin positive cells in the CN. Interestingly, in the same time calbindin positivity slightly decreased in the DCN and remained unchanged in the VCN, while calretinin positivity was not modified in either the DCN or the VCN (54, 55). The protective role of the Ca^{2+} binding proteins was further emphasized by those experiments in which healthy mice were subjected to strong sound stimuli (53). In these cases the increased acoustic nerve activity caused $[Ca^{2+}]_i$ elevations in the CN neurones with the same consequence as seen during the age-dependent progress of deafness, namely the number of calbindin and parvalbumin positive cells increased in positive correlation with the intensity of the noise exposure.

Role of the intracellular Ca^{2+} stores

There is still a considerable uncertainty regarding the function of the intracellular Ca^{2+} stores in neurones. It was proposed earlier that so-called peripheral nerve cells (located, for example, in sensory ganglia) possess stores filled with Ca^{2+} at rest. On the contrary, Ca^{2+} stores of neurones located centrally (e.g. in different nuclei of the brain) are empty at rest and these stores serve primarily as effective clearance tools when Ca^{2+} enters the cells (132). In other words, in the peripheral neurones the stores release Ca^{2+} to activate Ca^{2+}-dependent processes while in central neurones they accumulate Ca^{2+} from the cytoplasm contributing this way to the protection of the cells against the toxic effects of excessive $[Ca^{2+}]_i$. Recently the above scheme has been challenged by establishing that stores of the central neurones may also contain Ca^{2+} at rest (58). In any case the possibility cannot be excluded presently that the Ca^{2+} content of the stores at rest is greatly influenced by the experimental conditions.

There are only a few data about the presence and function of intracellular Ca^{2+} stores in the neurones of the auditory pathway. IP_3 receptors were detected in the avian NM (61) as well as in the Purkinje-like and cartwheel cells of the rat DCN (113). IP_3 receptors were also found in the cartwheel cells of several other mammals but only in the body and dendrites of these cells while their axons and axon terminals were free of

IP_3 receptors (121). Ryanodine receptor based CICR was reported in NM neurones (61), here the Ca^{2+} release through both IP_3 and ryanodine receptors could be attenuated by metabotropic glutamate receptor activation (61) (see later). In isolated rat DCN pyramidal cells the CICR mechanism was tested by applying caffeine (42, 118). The results were somewhat contradictory, in some cells the central neuron-like behaviour (empty Ca^{2+} stores at rest) could be observed (118) while later on a more systematic analysis found healthy cells with filled Ca^{2+} stores at the beginning of experiments (42).

Ionotropic glutamate receptor-related Ca^{2+} signalisation in the CN and NM

Glutamatergic transmission is the most common excitatory synaptic pathway in the CNS, and it is known that both the endings of the incoming primary sensory fibres and many of the interneuronal connections in the CN are also glutamatergic. It is plausible, consequently, to suppose that glutamate receptors fulfill important roles in the regulation of the neuronal activities in the CN complex.

EPSPs in glutamatergic synapses are elicited by the activation of ionotropic receptors. The early classification of these receptors (Ca^{2+} permeable NMDA and Ca^{2+} impermeable non-NMDA types) has been modified in the past few years as more and more details of the molecular structure of the receptors were revealed. Currently the GluR1-4 proteins are considered as AMPA-receptors, GluR5-7, KA1 and KA2 proteins are called kainate receptors while NMDA-receptors also proved to be heterogenous (NMDAR1, NMDAR2A-D) (49). In the case of the AMPA and kainate receptors the situation is further complicated by mRNA editing and by the presence of the flip-flop modules. The latter structural characteristics influence the kinetic features and Ca^{2+} permeability of the receptor channels (49).

Several studies were performed to clarify the glutamate receptor distribution in the CN and NM. Most reports seem to agree that GluR2-4 receptors are generally present in different mammals (64, 66, 101, 103, 127, 145) while the expression of the GluR1 receptor is more restricted (101, 127, 161). A similar distribution was found also in the chicken auditory pathway (107). There are no data about the presence of kainate receptors. From the NMDA receptors type 1 was found generally while the distribution of the type 2 variations was more limited (101). On the basis of the glutamate receptor distribution the conclusion was drawn that only AMPA receptors play major roles in auditory synaptic transmission (124). It has to be noted, however, that in the avian NM metabotropic glutamate receptor activation was reported to increase the $[Ca^{2+}]_i$ more effectively than the activation of the ionotropic receptors indicating that metabotropic receptors might be also involved in the information transfer here (164). At any rate, in mammals the role of the ionotropic glutamate receptors seems to be decisive.

Beyond mapping the receptors, the description of their characteristics was also attempted. It was noted that although GluR2 could be detected, its expression was noteworthly low compared either to the density of other AMPA receptors in the auditory pathway (103, 127, 145) or to the density of GluR2 in brainstem structures outside the auditory pathway (107). As the presence of the GluR2 protein yields Ca^{2+} impermeability to the AMPA receptor, its relatively low expression in the central auditory neurones results in considerable Ca^{2+} entry whenever glutamatergic transmission is activated. It is not surprising, therefore, that mainly non-NMDA ionotropic receptors might be responsible for the development of Ca^{2+} transients on glutamate application (68) and the Ca^{2+}/Na^{+} permeability ratio was estimated approximately 5 in these receptors (98). Similar results obtained in mammals gave some hints regarding the significance of this relatively high Ca^{2+} permeability. It was found that in rat the appearance and increasing density of the Ca^{2+} permeable AMPA receptors took place between the 4^{th} and 18^{th} postnatal days in correlation with the maturation of the hearing function (5, 14). This observation raises the possibility that the AMPA receptor mediated $[Ca^{2+}]_i$ increases may be important for the development of the synaptic connections.

When considering the developmental role of the Ca^{2+} permeable AMPA receptors, it seems to be an intriguing question why the NMDA receptors cannot fulfill this task? The key to this question may be the faster kinetics of the AMPA receptors, especially of those present in the neurones of the auditory pathway. The fast kinetics and rapid desensitisation of the AMPA receptors is characteristic, for example, of the glutamatergic synapses between the endings of the cochlear nerve fibres (endbulbs of Held) and their target neurones (bushy cells) in the VCN (37, 127). It was also shown that neurones receiving only direct acoustic nerve input have fast EPSPs while neurones receiving both acoustic nerve and parallel fiber inputs produce slower AMPA receptor-related EPSPs (37). Octopus cells of the gerbil, that show low GluR2 expression, have excitatory postsynaptic currents (EPSCs) with approximately 1 ms lifetime while in the cartwheel cells of the same species (in which GluR2 expression was more intense) the EPSCs lasted 100 ms (127). The characteristically short duration of the AMPA receptor-related EPSPs seems to be important in detecting the synchronisation of converging inputs (91) and in phase locking (106).

An observation of general importance was also made when the distribution of glutamate receptors in the DCN was studied. It was first noted that in the DCN pyramidal cells the GluR4 protein was present only at the basal dendrites where the acoustic nerve fibers terminate (115). Later on it was proved that the intracellular occurrence of the glutamate receptors in this cell type corresponds precisely to the exposition of the same receptors in the postsynaptic membranes, indicating that a specific targeting mechanism of the receptor proteins is available as soon as these

proteins are synthesised (116). It has been described recently that not only AMPA receptors but metabotropic glutamate receptors also exhibit uneven distribution in the apical and basal dendrites of the DCN pyramidal neurones (101). These findings support the view that site directed transport of proteins works in the neurones making possible to regulate and to change the functional characteristics of their surface membrane (8).

Metabotropic glutamate receptors in the CN and NM

Besides transmitting action potential coded information, glutamatergic synapses can profoundly influence the activity of the postsynaptic neurone via the Ca^{2+} entering through ionotropic receptors. The other way to modify the activity of the neurones by glutamate is its binding to metabotropic receptors. Currently three groups of these receptor proteins are distinguished. Group I contains mGluR1 and 5, group II is formed by mGluR2 and 3 while the remaining subtypes (mGluR4, 6, 7, 8) form group III. The groups can be characterized by their affinities to different agonists and antagonists (27).

Several successful efforts were made to detect mGluRs in CN and NM neurones. A great body of evidence refers to the presence of mGluR1 in the DCN of several mammals (101–103, 131, 154). Some of these data indicate that this subtype is the dominant mGluR form in this part of the nucleus (101). Expression of mGluR1 was described in cartwheel cells (103), in UBCs (56, 154), especially in the non-synaptic appendages of this cell type (56) as well as in pyramidal neurones (101, 115). Interestingly, in pyramidal cells mGluR1 seems to be localized only in the basal dendrites where the highly Ca^{2+} permeable AMPA receptors can also be found (101, 115). As mGluR1 activation may attenuate $[Ca^{2+}]_i$ increases (see later), this observation may suggest a protective role of mGluR1 against the Ca^{2+} entry caused by acoustic stimulation. Nevertheless, this conclusion is somewhat opposed by a paper describing mGluR1 also in the apical dendrites of pyramidal neurones (7). The occurrence of mGluR5, the other group I metabotropic glutamate receptor was less intensively tested. At any rate, its absence in UBCs and Golgi interneurones of the DCN was reported (56).

As for group II, mGluR2 was frequently detected in the DCN (88, 94, 101, 103), especially in the UBCs and Golgi neurones (94, 101, 103), while mGluR3 positivity was also characteristic of DCN, UBCs and Golgi cells (88). Similarly to mGluR1, UBCs express both group II receptors on their non-synaptic appendages (56, 85). Somewhat contrary to the above data only weak mGluR3 mRNA level in the CN was published (95).

There are only a few reports about the presence of group III receptors in the auditory pathway. One paper claims that mGluR7 is more widely distributed in CN than

mGluR4 indicating the presence of both subtypes (93). Other authors described an age-dependent expression of mGluR7 in the DCN with a peak density at about the 7th postnatal day (10). It is noteworthy that similar age-dependence of mGluR4 expression at the endings of the bushy cells in the MNTB (calices of Held) was also noted (26).

The presence of the mGluRs in the auditory pathway of several species raises the possibility that these receptors may influence the activity of different neurone types. A big amount of somewhat controversial data refer to the roles of mGluRs in the avian NM. Activation of mGluRs here attenuates the depolarization-elicited increases of $[Ca^{2+}]_i$ (69) as well as caffeine induced Ca^{2+} transients (60, 61). The latter effect could be evoked also by t-ACPD indicating the primary involvement of group I receptors. In line with these data glutamate was reported to decrease IP_3 production and Ca^{2+} release via mGluRs (61). In contrary to the above findings, facilitation of IP_3 formation by glutamate and t-ACPD was also described in NM neurones (166) together with mGluR induced $[Ca^{2+}]_i$ increases in the absence of extracellular Ca^{2+} (164). A possible explanation of the conflicting statements may be yielded by the observation that mGluR1 expression and, consequently, the modulation of intracellular Ca^{2+} homeostasis via these receptors show age dependence (162).

There are only a few reports on direct mGluR actions under acute experimental conditions in mammalian CN. In guinea pig the effects of parallel fiber stimulation were inhibited by t-ACPD, a group I agonist, in DCN cartwheel cells but not in pyramidal neurones, while groups II and III agonists did not show this action (79). In cats and gerbil mGluR agonists increased sound-evoked discharge rates of CN neurones in a short run, while on a long run both depression and potentiation could be observed (125). These data seem to indicate that mGluRs may modulate the transmission of the auditory information in mammals, too. The role of these receptors, however, was more obviously shown in chronic experiments and under non-physiological (or even pathological) conditions.

It is generally accepted that there is an early and marked increase of the $[Ca^{2+}]_i$ in avian NM neurones following deafferentation (i.e. terminating the excitatory input via the 8th cranial nerve by any method). The Ca^{2+} accumulation could be prevented by increasing protein kinase C or protein kinase A activity and by the orthodromic electrical stimulation of the acoustic nerve (165). As the protective effect of the 8th nerve stimulation could be suspended by mGluR antagonists (167) the conclusion was drawn that under normal conditions the acoustic nerve activity ensures the balance of the cytoplasmic Ca^{2+} homeostasis via mGluRs. After deafferentation this balance is deteriorated and the $[Ca^{2+}]_i$ increases. The other modifications seen after deafferentation, including decreased NM cell size, degeneration of these neurones (142), deterioration of cytoskeletal proteins despite normal mRNA levels (63) are either the consequences of the increased $[Ca^{2+}]_i$ or develop independently. Interestingly,

calretinin expression seems to be unchanged following deafferentation (138). This finding may be explained by supposing that the two mechanisms causing opposite changes of the calretinin expression (decrease due to the missing mGluR influence and increase due to the elevated $(Ca^{2+})_i$) counterbalance each other's effect.

Deafferentation experiments in mammals yielded similar results. In the rat VCN modifications in the electrophysiological parameters of the neurones were noted (33), in rat VCN and DCN c-fos transciption decreased (62). In VCN neurones of the gerbil deafferentation decreased protein synthesis within 2 hours, a decrease of the cell size was observable 2 days later (133). Following deafferentation of the CN in cat, stimulation of the 8th cranial nerve increased the survival of the neurones in the VCN but not in the DCN (76). Besides experimental deafferentation, mammals suffering from genetic deafness also showed modifications that could be regarded as the consequences of the reduced or missing mGluR action. In mice the neuronal deterioration was especially pronounced in those layers of the DCN where the acoustic nerve fibres terminate (150). Morphological changes of the VCN octopus cells were also reported (149). Moreover, the endings of the acoustic nerve fibres (the endbulbs of Held) became smaller and had modified structure (71). In genetically deaf cats smaller endbulbs and globular cells were found, too (108, 120). In these cats a positive correlation was established between the extent of the morphological changes of the endbulbs and the severity of the deaf state (122). Finally, in deaf human patients a decrease of both the CN volume and the neuronal somata size in the CN was published (130).

A plausible control of the above observations may be the investigation of the consequences of strong stimulation of the auditory pathway. When this experiment was performed in chicken by using sustained electrical stimulation of the 8th cranial nerve, increased ribosomal RNA staining of the NM neurones was found provided that the stimulation was presynaptic and the postsynaptic transmitter action was not blocked (52). These findings indeed seem to justify the deafferentation results. In rat, however, 2 hours long noise load decreased the cross-sectional area and the thickness of the postsynaptic density at the endbulb of Held/bushy cell synapses (109) which is difficult to reconcile with the results of the experiments employing deprivation of sensory inputs.

Concluding remarks

As it has been pointed out earlier, neurones, especially those located in the spinal cord and in different brain regions, only recently became accessible for modern experimental techniques. It is not surprising, therefore, that in different particular fields of neurobiology the mass of the relevant data is rapidly increasing. This statement is

also true for the problems of auditory information processing. Nevertheless, some conclusions can be drawn and the most immediate further steps of the future work can be specified.

It is generally accepted that the CN complex, and the DCN in particular is very strictly ordered. The high level of structural organisation must certainly serve some important functions, although the experimental data collected so far are not sufficient to allow complete understanding how exactly the signal processing in the CN complex occurs. However, a few interesting and noteworthy points emerge when one considers some important findings of the hitherto conducted investigations.

It seems that some neurones of the CN are "designed" to achieve very high fidelity transmission of the auditory information. These cells can maintain and pass on the temporal coding of the acoustic information. The most prominent ones in this regard are the bushy and the octopus cells, and it seems that there are several features which ensure that they are capable of fulfilling this task.

i) These neurones receive information from the thick, myelinated, hence rapidly conducting (or type I) acoustic nerve fibres.

ii) The synapses (e.g. endbulbs of Held) formed by the acoustic nerve fibres and the second-order sensory neurones are glutamatergic and the composition of the ionotropic glutamatergic receptors here (low expression level of GluR2 protein) makes possible the development of short EPSPs allowing high frequency information transfer.

iii) The membrane properties of bushy and octopus cells allow rapid action potential firing if sufficiently strong depolarization stimulates them. It is particularly interesting to note that the rapidly responding character of different cells seems to be achieved by similar membrane conductances, keeping the membrane time constant sufficiently low. Investigators reported on the presence of a hyperpolarization-activated non-specific cationic conductance (I_h) as well as on the activity of a low threshold K^+ current in both types of cells. It is also interesting to note that the h-current could be modulated in both cases, indicating that the responsiveness of these cells can be altered.

iv) The axons of the bushy and octopus neurones are fairly thick, hence the information provided by these neurones can reach the target cells and projection areas relatively rapidly, completing the requirements for high fidelity transmission.

Despite the above similarities there are some significant differences as well between these two cell types. The highly specific and unique synaptic apparatus of the bushy neurones is also a factor that contributes to the faithful temporal coding of incoming information, and these cells produce a "primary-like" firing pattern. It seems to be reasonably certain that this high degree of temporal precision is important for the sound source localisation. On the other hand, octopus cells lack such highly specialised synaptic apparatus, and they are more likely to be involved in recognising the

coincidence of firing of several acoustic nerve fibres, and can provide information about the onset of this coincidence.

Some (i.e. stellate and pyramidal) cells of the CN receive information via the thick acoustic fibres, but these cells have only relatively thin axons, and it was proposed, therefore, that in the function of these cells the timing of the information is not so vital. While stellate cells produce a non- or slowly adapting response, the pyramidal cells can produce a variety of response patterns on stimulation. Although it does seem to be clear that the different activity patterns are determined by the prior membrane potential of these neurones, and hence by the availability of K^+ channels on activation, the actual function of these cell types is still not clear. It seems particularly interesting to consider that a great diversity of voltage-gated Ca^{2+} channels has been reported in the pyramidal cells, which may alter the firing characteristics and membrane properties of these neurones. Moreover, possible changes caused by the entry of Ca^{2+}, and the subsequent increase of the $[Ca^{2+}]_i$ may also affect the basic functional characteristics. It is tempting to think that the various Ca^{2+} channels may provide targets for neuromodulatory mechanisms.

In the case of the pyramidal cells the variability of the activity patterns may reflect also the dynamism of their inputs. As it was mentioned, these neurones accept direct acoustic information as well as a more complex input via the parallel fibre system and their activity state may reflect an interaction of the two inputs. This idea is supported by the view that some cells in the CN are even more clearly usable for intermodal integration. Two interneurones have been specifically associated with such a function, namely the Golgi and the granule cells. Both cell types receive information from the acoustic fibres (although in a number of cases this input is either indirect or is mediated via the slowly conducting type II acoustic nerve fibres). Granule cells are also contacted by fibres of the cuneate nucleus, allowing simultaneous processing of acoustic and somatosensory information. As Golgi cells and granule cells are mutually connected to each other, and both cell types act as interneurones of the CN and contact several other cells, it is likely that they can significantly influence the activity of their targets. Although both granule and Golgi cells have unusually high input resistances, making them extremely sensitive to stimulation, they are also rather different in their functions as one of them is an excitatory and the other one is an inhibitory interneurone.

Another rather prominent interneurone of the CN is the cartwheel cell population. Cartwheel cells seem to be identical with the Purkinje cells of the cerebellum, as they express similar antigens and there are similarities in their synaptic organisation as well. On the basis of these similarities it can be postulated that cartwheel cells must play essential roles in modifying the activity of the efferent neurones of the CN, but their exact role is still to be defined.

There are some other cells in the CN, where the information about their functional properties is still lacking, and hence their role and function in the signal processing is still uncertain. In the cases of the giant cells, the authors agree that their huge receptor field makes them capable of collecting information from numerous acoustic fibres, but the significance of this finding is not really clear yet. In the cases of the fan, star, radiate and vertical cells the functional data are so insufficient that it is impossible even to suggest what role they may serve in the activity of the CN.

The processing of the auditory information primarily involves the generation, conduction and transmission of the action potential trains. As it has been indicated, however, these "pure" electrophysiological events are modulated by several regulatory mechanisms. Although the number of the involved intracellular signalisation pathways may be high, relatively small pieces of information are available about the details of their function. Nevertheless, an especially intriguing and relatively widely studied topic is the relation of the $[Ca^{2+}]_i$ to the neuronal activity. The data obtained so far suggest a rather close connection of glutamatergic transmission and the regulation of the $[Ca^{2+}]_i$. It can be postulated with certainty that the rather high frequency synaptic transmission and action potential firing are accompanied by rather significant Ca^{2+} loads of some of the CN neurones. Not surprisingly, these neurones possess effective defense mechanisms against Ca^{2+}-related excitotoxicity. On the other hand, the cells seem to be adapted so "perfectly" to the incoming activity that the loss of this input leads to the collapse of their intracellular Ca^{2+} homeostasis and to their eventual destruction. The understanding of this complicated regulatory system requires further efforts to study the role of the voltage-gated Ca^{2+} channels and ionotropic glutamate receptors in the activity-dependent Ca^{2+} loading, to analyse the role of the metabotropic glutamate receptors in the protection against Ca^{2+}-related neurotoxicity and to explore the role of the metabotropic glutamate receptors in transmitting trophic influence.

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