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Review Article

The cerebellar (para)flocculus: A review on its auditory function and a possible role in tinnitus

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ABSTRACT

The cerebellum is historically considered to be involved in motor control and motor learning. However, it is also a site of multimodal sensory and sensory-motor integration, implicated in auditory processing. The flocculus and paraflocculus are small lobes of the cerebellum, in humans located in the cerebellopontine angle. The last two decades, both structures have been a subject of interest in hearing loss and tinnitus research. The current review summarizes insights on the auditory function of the (para)flocculus and its contribution to hearing loss and tinnitus. This leads to the hypothesis of a feedback loop between the paraflocculus and the auditory cortex. Disruption of this loop may be instrumental in both maintaining tinnitus and reducing tinnitus. Although the research mostly has been performed in animals, the implications in humans are also discussed. If the (para)flocculus indeed comprises an auditory function and is part of a tinnitus-mechanism, this would potentially open up new treatment options that involve direct intervention at the (para)flocculus.

1. Introduction

The cerebellum has historically been considered to be involved in motor control and motor learning. However, currently an increasing awareness exists that the cerebellum may also be involved in other neural pathways. The embryologic origin of the cerebellum is the alar plate, the dorsal half of the neural tube, which is the source of sensory structures. Presumably due to this origin, the cerebellum might also be a site of multimodal sensory and sensory-motor integration (Herrup and Kuemerle, 1997; Voogd and Wylie, 2004). Although less acknowledged, the cerebellum receives both direct and indirect auditory input as shown in animal studies. The cochlear nucleus projects directly to the vermis (Huang et al., 1982) and the lateral cerebellar nucleus (Wang et al., 1991). Indirectly, the cerebellum receives auditory input via the pontine nuclei from the inferior colliculus (IC) and the auditory cortex (Aitkin and Boyd, 1978; Brodal and Bjaalie, 1992; Kawamura, 1975; Kawamura and Chiba, 1979; Schmahmann and Pandya, 1991).

The flocculus (FL) and paraflocculus (PFL) are small cerebellar lobes, in humans located in the cerebellopontine angle, that are known to have a strong relation with the vestibular system. In line with the auditory connections of the cerebellum, the FL and PFL have been implicated in hearing loss and tinnitus, the phantom perception of sound without an external acoustic stimulus. If the FL and/or PFL indeed comprise an auditory function, this could very well be a new area of interest with regard to tinnitus treatment. Therefore, this review aims to provide an overview of current knowledge on the auditory function of the FL and PFL. First, the well-known functions and neural connections of the FL and PFL are discussed. Second, because almost all studies on the FL and PFL regarding hearing loss and tinnitus are performed in animals, similarities and differences between non-human mammal and human anatomy and function are elaborated on. Third, to be able to interpret studies about the auditory function of the FL and PFL, a brief overview on basic cerebellar cytoarchitecture and circuitry is pro-

vided. Last, auditory neural connections of the FL and PFL and their potential role in hearing loss and tinnitus are highlighted.

2. The (para)flocculus and comparative cerebellar anatomy

2.1. (Para)floccular anatomy and function

In humans, the FL is a small lobe of the cerebellum, situated on both sides at the posterior border of the middle cerebellar peduncle, anterior to the biventral lobule. It consists of a rosette-like cluster of approximately fifteen folia (Tagliavini and Pietrini, 1984). Dorsally, the accessory paraflocculus (acPFL) is located, which is on average 40% the size of the FL (Løyning and Jansen, 1955). However, the size and morphology of the PFL varies greatly from a small flattened lamella to a rosette-like cluster of folia similar to the FL (Tagliavini and Pietrini, 1984). The FL and the nodulus of the vermis together form the flocculonodular lobe, a.k.a. the archicerebellum or vestibulocerebellum (Roostaei et al., 2014).

The flocculonodular lobe is recognized for its strong relation with the vestibular system. It receives vestibular afferents from the vestibular nuclei and directly from the vestibular nerve (Roostaei et al., 2014). The main projections of the FL are to the superior vestibular nucleus, the medial vestibular nucleus and the posterior interposed nucleus (de Zeeuw et al., 1994). The PFL receives strong (visual) input from the pontine nuclei, with a much stronger projection in the dorsal PFL than in the ventral PFL (Azizi et al., 1985; Azizi and Woodward, 1990; Burne et al., 1978; Glickstein et al., 1994). The FL and PFL are essential in the vestibular-ocular reflex, in which compensatory eye movements are generated to maintain gaze on a target during head motion. They are crucial to vestibular-ocular reflex gain and direction, pulse-step matching for saccades, pursuit gain, and gaze-holding (Beh et al., 2017). However, although both are involved, the relative role of the FL versus PFL in vestibular function, gaze-holding and smooth pursuit is disputed (Belton and McCrea, 2000; Nagao, 1992; Rambold et al., 2002).

2.2. Comparative anatomy of the cerebellum and (para)flocculus

To be able to (inter)compare animal study results and to extrapolate to humans, one should be acquainted with the similarities and differences in cerebellar anatomy within different species. Since all studies addressing auditory function of the (para)flocculus are performed in mammals, non-mammalian animals on this topic are disregarded. The basic structure of the cerebellum is highly comparable within mammals, except for some deviations discussed later. The cerebelli of mammals all consist of two hemispheres that are foliated with a complicated pattern (Voogd and Glickstein, 1998). The anterior lobe and the lobulus simplex of comparative anatomy comprise a single unbranched chain of folia, which divides into two folial chains of the hemispheres and the folial chain of the vermis in the middle. The folial chains of the hemispheres consist of two folial loops: one in the ansiform lobule (crus I and crus II) and one in the PFL. The last section of these chains turns back on itself and is called the FL (Fig. 1) (Squire, 2009). The output of the cerebellar cortex is arranged in longitudinal zones, parallel to the long axis of the folial chains. Purkinje cells (the only cerebellar output neurons) belonging to a specific zone project to a particular cerebellar or vestibular target nucleus. Vice versa, the afferent projections are arranged according to the same principle. For instance, the olivocerebellar projection: a specific subnucleus projects to a single Purkinje cell zone or to a pair of zones that in turn target the same nucleus (Voogd and Glickstein, 1998). This zonal pattern is very similar across mammals and therefore, the subdivision of cerebellar nuclei is similar in all mammalian species

(Nieuwenhuys et al., 1998). It can be concluded that basic cerebellar anatomy and its projections are comparable between mammalian species and that it, specialized functions aside, is plausible to extrapolate outcomes of mammalian studies to humans.

Nevertheless, there may be an exception. The following was stated in 1947 by Larsell, an anatomy professor who laid the groundwork of modern knowledge about cerebellar anatomy: *"The paraflocculus of comparative anatomy includes the human tonsilla and lobulus biventer, or at least their lateral extremities. (…) The human paraflocculus, or accessory flocculus, has no relation to the paraflocculus, so-called, of mammals, on the one hand, or to the flocculus, on the other, save proximity to the latter. It is derived from the region of the tonsilla"* (Larsell, 1947). The homology of the mammalian PFL with the human tonsil is mainly based on the development of their limiting fissures and receives support from the presence of a folial loop in this region (Voogd, 2003). Although still a matter of debate, nowadays the following homologies are used: non-human mammalian FL and human FL, non-human mammalian ventral PFL and human acPFL and non-human mammalian dorsal PFL and human tonsil (Fig. 1). Some authors also classify (part of) the biventral lobule as the counterpart of the non-human mammalian dorsal PFL (Voogd and Ruigrok, 2012).

Consequently, translating from non-human mammals to humans requires differentiation of the ventral and dorsal PFL in animal studies. In non-human mammals the border between the ventral and dorsal PFL is arbitrary and it occupies a dissimilar position in different species (Voogd and Barmack, 2006). Differences in (para)floccular sizes between mammals are probably caused by variations of cerebellar cortex zone width (Voogd and Glickstein, 1998) and the presence or absence of certain zones (Voogd and Barmack, 2006). This could explain the major differences in FL and PFL sizes between species: in some species the PFL is bigger than the FL and vice versa. Even within humans the morphology and size of the FL and especially the acPFL is highly variable, although it is not known if this also relates to its function (Tagliavini and Pietrini, 1984).

3. Cerebellar signal processing

3.1. Classic cerebellar cytoarchitecture and circuitry

Many of the animal studies on the (para)floccular auditory function identified the properties of individual neurons. Thus, one should be acquainted with cerebellar cytoarchitecture and its circuitry. The basic plan of the cerebellar cortex, including the (para)flocculus, is similar across vertebrates (Voogd and Glickstein, 1998). The cerebellar cortex consists of three layers, from the superficial molecular layer to the medial Purkinje cell layer and the inner Granule cell layer (Fig. 2). Each layer houses its own set of cells. The major input to the cerebellar circuitry enters via the excitatory mossy fibres and climbing fibres. To a lesser extent, input enters the cerebellar cortex via diffusely organized mono-aminergic and cholinergic afferents (Glickstein et al., 2011; Martin, 2012; Roostaei et al., 2014; Voogd and Glickstein, 1998; Voogd and Wylie, 2004). Climbing fibres originate from the contralateral inferior olivary nucleus. They synapse on the cerebellar nuclei and on dendrites of the inhibitory Purkinje cells in the molecular layer. Purkinje cells are the only output neurons of the cerebellar cortex. They project to the cerebellar nuclei, in which input from mossy fibres, climbing fibres, and Purkinje cells is merged into an output. These nuclei form the output of the cerebellum, projecting towards the brainstem and thalamus. The only exception is the vestibulocerebellum. Fibres from Purkinje cells originated in this lobe project directly to the vestibular nuclei, bypassing the cerebellar nuclei.
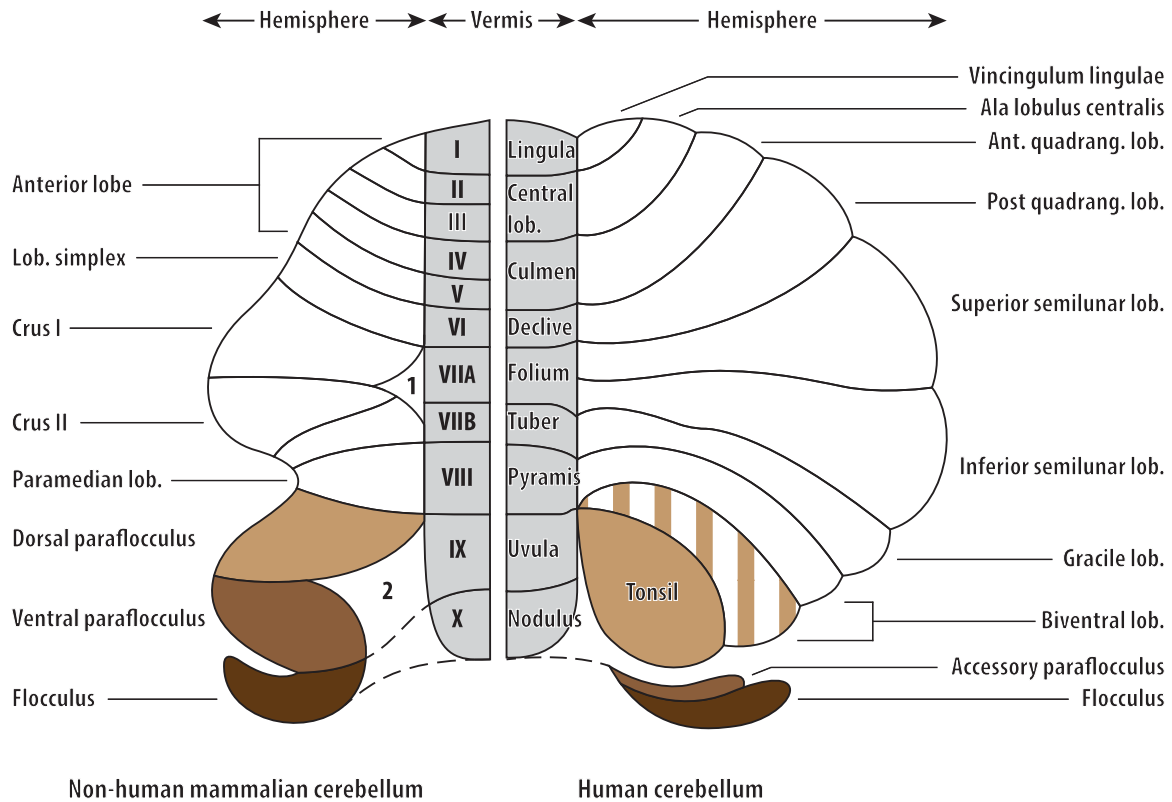
Fig. 1. Comparative neuroanatomy of the non-human mammalian and human cerebellum. Comparative nomenclature for the non-human mammalian cerebellum is depicted on the left and for the human cerebellum on the right. The anterior lobe and lobulus simplex consist of a single folial chain that branches just behind the lobulus simplex into one folial chain of the vermis and two folial chains of the hemispheres. The latter two consist of two loops, one in crus I and crus II (1) and one in the PFL (2). The last section turns back on itself (the FL). The non-human mammalian FL, ventral PFL and dorsal PFL are the homologues of the human FL, acPFL and cerebellar tonsil, respectively. Some also include (part of) the human biventral lobe as the counterpart of the non-human mammalian dorsal PFL. Modified after Voogd and Glickstein (1998) and Voogd and Ruigrok (2012).
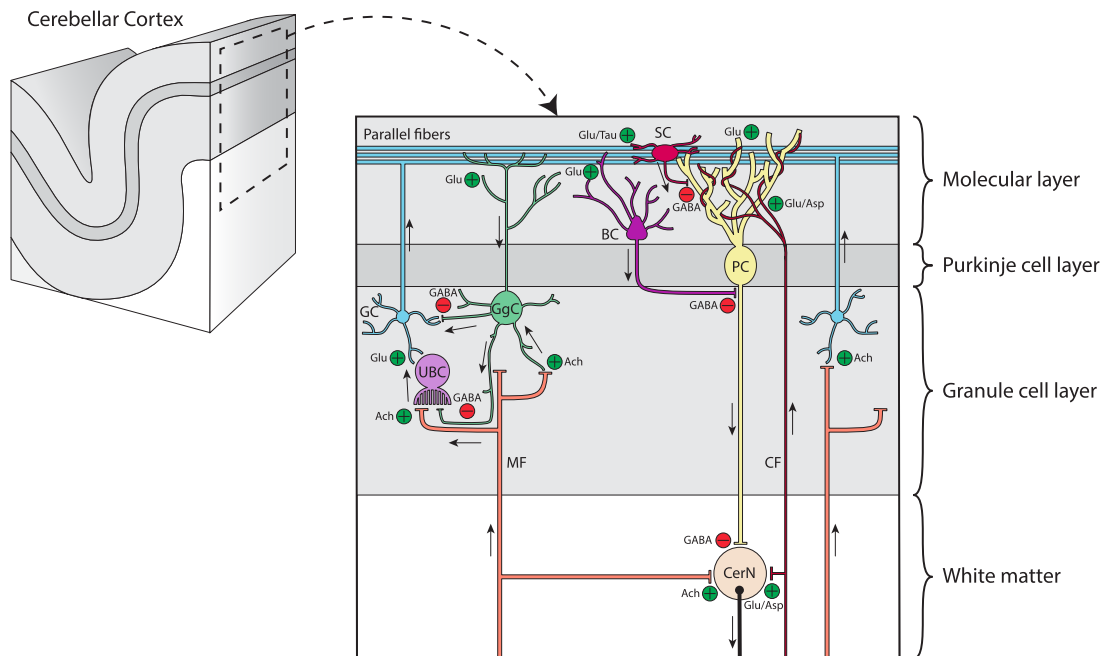
Fig. 2. Simplified cerebellar cortical circuitry. The cerebellar cortex consists of three layers: the Molecular layer, Purkinje cell layer and Granule cell layer. In these layers Unipolar Brush cells, Golgi cells, Granule cells, Basket cells, Stellate cells and Purkinje cells reside. Mossy fibres and Climbing fibres form the mayor input of the cerebellum. At synapses involved neurotransmitters are depicted and whether they are excitatory or inhibitory. Abbreviations: Ach, acetylcholine; Asp, aspartate; BC, Basket cell; CF, Climbing fibre; CerN, cerebellar nucleus; GABA, γ-amino butyric acid; GC, Granule cell; GgC, Golgi cell; Glu, glutamate; MF, Mossy fibre; PC, Purkinje cell; SC, Stellate cell; Tau, taurine; UBC, Unipolar Brush cell. Modified after Dufor (2017).
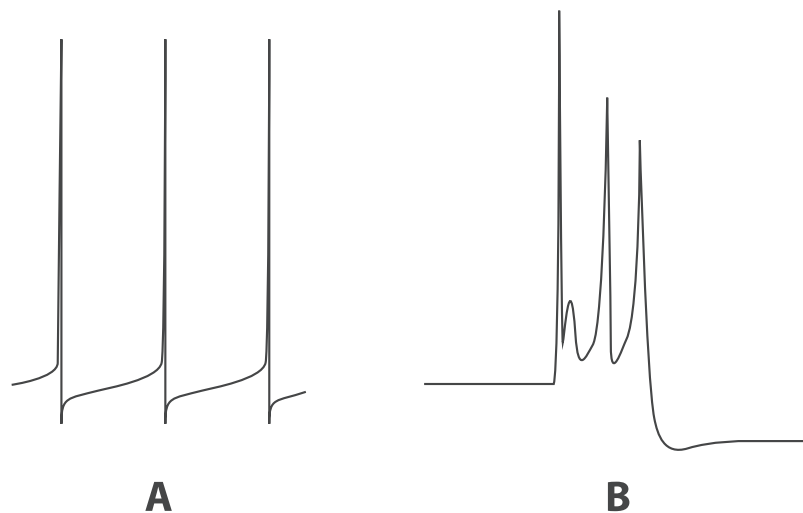
Fig. 3. Firing pattern of Purkinje cells. (A) Simple spikes, produced as a result of Mossy fibre input and intrinsic activity of Purkinje cells. (B) Complex spikes, produced as a result of Climbing fibre input.

Mossy fibres originate from many different structures, including the pontine nuclei, spinal cord, vestibular nuclei and the reticular formation. They form synapses with excitatory Granule cells and interneurons in the granular layer of the cerebellum (Unipolar Brush cells (UBC) and Golgi cells). Axons of Granule cells ascent into the molecular layer and bifurcate forming parallel fibres in a distinct T-shape. These fibres form excitatory synapses with the dendritic trees of the Purkinje cells. The parallel fibres also synapse on the inhibitory Golgi cells, Stellate cells, and Basket cells, of which the latter two provide feed-forward inhibition to Purkinje cells. Golgi cells form an inhibitory connection to Granule cells and UBCs, providing feed-backward inhibition (Glickstein et al., 2011; Martin, 2012; Roostaei et al., 2014; Voogd and Glickstein, 1998). Altogether, signal processing in the cerebellum is almost entirely feedforward; a signal goes from input to output unidirectionally with little recurrent internal transmission.

3.2. Cerebellar action potentials

Purkinje cells, the output neurons of the cerebellar cortex, generate two types of electrical behaviour: simple spikes (Fig. 3A) and complex spikes (Fig. 3B). Simple spikes are single action potentials. Their firing pattern is based on both the input of parallel fibres and interneurons of the molecular layer to Purkinje cells, and on the intrinsic activity of Purkinje cells itself. Therefore, simple spikes are continuously produced, even without synaptic input. In resting animals, they occur at rates ranging from 40 to 100 spikes per second. This allows the Purkinje cells to tonically inhibit their target neuron. Complex spikes are burst responses to very large excitatory synaptic input originating from Climbing fibres, which typically average 1 Hz. They inhibit simple spike firing, leading to either fewer simple spikes immediately after a complex spike, or to a period reset in which simple spike firing is phase shifted (Gruol et al., 2016).

3.3. Auditory involvement

Auditory connections can be assessed using two different methods. The first is by using neural tracing studies to identify anatomical connections. The second is by using electrophysiological measurements, to determine the functional connection of one structure to another.

3.4. Anatomical connections

A method commonly used to study neuroanatomical connections is the injection of horseradish peroxidase (HRP). HRP is a plant enzyme that enters axons and is transferred back to cell somata by active, retrograde transport (Köbbert et al., 2000). It therefore can be used for retrograde neural tract tracing. For anterograde tract tracing amino acids are used (Lanciego and Wouterlood, 2011). Neurons take up amino acids and incorporate them in their polypeptide macromolecules. Some of these are transported to the axon terminals, enabling anterograde tracing of the axons.

Neuroanatomical literature on the FL and PFL with regard to auditory function is sparse. Studies on this topic have only been performed in rats (Azizi et al., 1985; Burne et al., 1978; Shute and Lewis, 1965) and chinchillas (Morest et al., 1997). Injection of the anterograde tracer ^{3}H-amino acid in the secondary auditory cortex of rats showed labelling of the lateral, rostral pons (Fig. 4) (Azizi et al., 1985). The same area was labelled by injection of the retrograde tracer HRP into the PFL (Azizi et al., 1985). These conjoined results suggest that an auditory pathway exists from the secondary auditory cortex, via the pons, to the PFL. The pontine neurons are known to project sparsely to the ventral PFL, but extensively to the dorsal PFL, so this distribution may also exist with regard to this auditory pathway (Burne et al., 1978; Osanai et al., 1999; Voogd and Glickstein, 1998). Moreover, after lesioning the IC of bats Henson et al. (1968) showed degenerated fibres from the IC connected directly to the PFL and in the PFL portion of the dentate nucleus. The degeneration was localized almost entirely ipsilateral.

An auditory connection to the FL has also been demonstrated. An ascending branch from the cochlear nerve through the dorsal anterior ventral cochlear nucleus (AVCN) into the FL of chinchillas was established (Morest et al., 1997). In rats, the flocculonodular lobe also receives afferent fibres from the cochlear nucleus (Shute and Lewis, 1965). An efferent auditory connection of the FL and PFL has not yet been described.

4. Electrophysiological projections

4.1. Electrical simulation

Azizi et al. (1985) were the first to examine PFL responses to electrical stimulation of the primary and secondary auditory cortex. Stimulation of the contralateral auditory cortex elicited a response in 26% of PFL Purkinje cells in rats (Fig. 4). The response
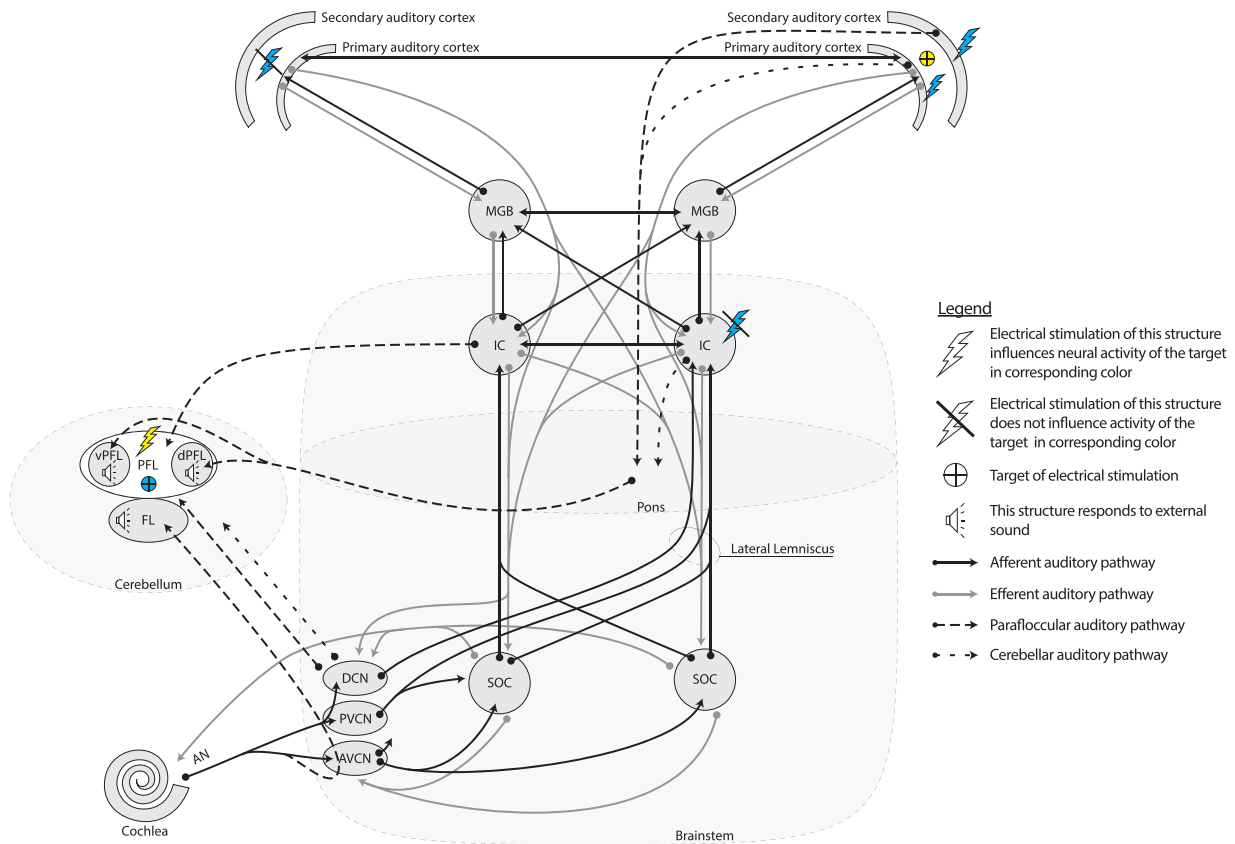
Fig. 4. Auditory connections. Depicted is a simplified version of the classical afferent and efferent auditory pathway, supplemented with cerebellar anatomical connections. Branching and merging of arrows do not necessarily mean that fibres branch or merge. Results of electrophysiological studies are depicted as well. Abbreviations: AN, auditory nerve; AVCN, anterior ventral cochlear nucleus; DCN, dorsal cochlear nucleus; dPFL, dorsal paraflocculus; FL, flocculus; IC, inferior colliculus; MGB, medial geniculate body; PFL, paraflocculus; PVCN, posterior ventral cochlear nucleus; SOC, superior olivary complex; vPFL, ventral paraflocculus.

consisted of a mixed response: 5–8 ms single or double spike excitation followed by a variable period of inhibition for Mossy fibre input. Mean latencies to the onset of excitation and inhibition were 9.5 ms ± 3 (SD) and 14 ms ± 2.4 (SD), respectively. At lower intensities of stimulation, only inhibitory responses were elicited in most of the responsive neurons. Often post-inhibitory rebound excitation of simple spike activity was seen, varying in magnitude and duration. More than half (57%) of interneurons, especially the smaller ones from the Molecular layer, responded to electrical stimulation. No evoked activity caused by Climbing fibre firing (complex spikes) was seen and therefore it could be stated with certainty that no Purkinje cells were measured. Stimulation of the secondary auditory cortex had the lowest threshold and elicited the most dramatic excitatory-inhibitory responses. The primary auditory cortex had a much higher threshold and only evoked inhibitory responses in the PFL. This is in line with the observation that the pontine projections arise mainly from the secondary auditory cortex and less from the primary auditory cortex (Kawamura and Chiba, 1979; Schmahmann and Pandya, 1991). Electrical stimulation of the contralateral inferior tectum (i.e. inferior colliculus) in rats did not elicit a response in the PFL, nor did electrical stimulation of the ipsilateral auditory cortex in bats (Azizi et al., 1985; Sun et al., 1990).

To evaluate the effect of the PFL on the auditory cortex, the PFL itself was stimulated in rats by Du et al. (2017), showing that 71.4% of neurons in the contralateral auditory cortex of rats responded to stimulation. There were two distinct responses: about half of the neurons showed an increase in firing rate and half of them a reduction. It was determined that a higher proportion of pyramidal neurons were excited than interneurons.

In conclusion, electrical stimulation of the auditory cortex affected neural responses in the contralateral PFL and vice versa. Responses from the PFL to electrical auditory cortex stimulation could be explained by a corticopontinecerebellar pathway as described in the anatomical studies. Although an efferent auditory connection of the PFL has not yet been described, these results indicate that there probably is one, perhaps indirect. Nevertheless, it is not known by which pathway this would be.

4.2. Auditory stimulation

The FL has been shown to respond to external sounds in cats (Marsh and Worden, 1964; Woody et al., 1999) and the PFL in monkeys (Mortimer, 1975), bats (Horikawa and Suga, 1986; Sun et al., 1990) and rats (Azizi et al., 1985; Azizi and Woodward, 1990). Auditory stimulation delivered by speakers showed alteration of firing rates in both Purkinje cells and interneurons of the PFL in rats. In approximately 20% of PFL Purkinje cells, alteration in spike activity in response to tone bursts was demonstrated. In only one out of seven Purkinje cells, this was frequency-specific for particularly the complex spike activity. At a frequency of 8 kHz, the complex spike activity was doubled, with much less or no change in response to frequencies. In 28% of non-Purkinje cells strong excitatory or inhibitory responses were shown; only three (3/16) showed frequency specific changes (Azizi et al., 1985). Unilateral noise bursts evoked distinct local field potentials (LFP) in 60% of recording sites in the contralateral PFL. The mean LFP consisted of four positive peaks at 10, 50, 100 and 200 ms after stimulus onset, with the latter two only present in 80– 90% of the samples. Approximately 20% (29/144) of recorded multiunit clus-

ters (MUC) produced an increase in firing rate in response to noise burst or tone burst. Out of 29 MUCs, 22 showed responses to both noise burst and tones, but three (3/29) only responded to noise and four (4/29) only to tones. Ten MUCs showed a clear increase in firing rate in 1–8 kHz with a peak response 15–20 ms after stimulus onset. Much weaker responses were present in 12.1–27.7-kHz stimulation (Chen et al., 2017).

In mustached bats, 89% and 98% of neurons responded to constant-frequency tones produced by a speaker in the dorsal and ventral PFL, respectively. None showed a facilitative response to a combination of two constant-frequency sounds, a combination of two frequency modulated sounds or noise bursts. Most PFL neurons showed small simple spikes and low rates of spontaneous discharges. The best frequency of most neurons was 25–26 kHz. Almost all neurons were responsive to 23–29 kHz. Latencies ranged from 15–30 ms and 12–40 ms in the dorsal PFL and ventral PFL, respectively. Furthermore, there were no indications that PFL auditory neurons were less sensitive to sound than peripheral auditory neurons (Horikawa and Suga, 1986). In horseshoe bats the best frequency of PFL neurons ranged from 34 to 68 kHz, mostly between 44 and 66 kHz. Response latencies ranged from 10 to 48 ms (21.04 ms ± 7.89 (SD)) (Sun et al., 1990). Latencies were longer in PFL neurons than in neurons of the cerebellar vermis, crus and hemisphere (Horikawa and Suga, 1986; Sun et al., 1990). This suggests that the auditory pathway to the PFL might be a different partway than to the cerebellar vermis, crus and hemisphere.

Sun et al. (1990) also studied the additional effect of electrical contralateral cortical stimulation on evoked acoustic responses in the PFL. They described that a facilitative effect was present. However, they did not describe the characteristics of PFL neurons specifically, but rather of representative cerebellar auditory neurons. Remarkably, when they applied topical procaine (local anaesthetic) on the auditory cortex, all responses in the PFL disappeared independent of the type of stimulation (i.e. only electrical cortical stimulation, only auditory stimulation or a combination of both). Thus, neural activity in the auditory cortex is required for a PFL-response to acoustic stimulation.

In cats, the response of the FL to clicks produced by a speaker was greater in cells with simple spikes than in cells with complex spikes. The onset of increased activity in simple spike cells was present in 8–16 ms after presentation of the click, with an increased activity of 24%. In complex spike cells activity increased with only 7% and the onset was later, at > 16 ms (Woody et al., 1999). Although sparsely studied, this suggests that not only the PFL, but also the FL, is responsive to sounds.

In conclusion, given the relatively long latency of 12–48 ms of PFL sound-evoked activity in bats, the PFL presumably receives its auditory input via a multisynaptic pathway. It appeared that PFL sound-evoked activity is dependent on contralateral auditory cortex activity. Therefore, we propose that the PFL mainly receives its auditory input from the contralateral auditory cortex via the pontine nuclei (Fig. 4). The latency of 9.5 ms of PFL activity in rats after electrical auditory cortex stimulation fits in this theory and it is in accordance with the found anatomical auditory connection between the auditory cortex and the PFL. The functional role of the input from the ipsilateral IC to the PFL is not clear.

5. The paraflocculus in hearing loss and tinnitus

Acquired hearing loss and tinnitus are strongly interrelated. They share major causes such as traumatic noise exposure and ototoxic drugs, and both can lead to tonotopic map alterations, changes in spontaneous firing rates (SFR), and neural synchrony (Baguley et al., 2013; Eggermont, 2017; Koops et al., 2020). It is no surprise that hearing loss and tinnitus often exist alongside. Additional to the complicated pathophysiology, an additional challenge

in studying tinnitus and hearing loss is to separate the effect of both. This is especially true in animals, since methods to indicate the presence of tinnitus are not trivial and techniques to induce hearing often also cause tinnitus (Brozoski and Bauer, 2016).

5.1. Hearing loss

Only a few studies on the role of the FL and PFL in hearing loss have been published. A significant increase in mRNA levels of glutamate decarboxylase 1 (GAD1) was present in the ipsilateral PFL, two weeks after both unilateral acoustic and mechanical trauma in guinea pigs (Mulders et al., 2014). GAD is an enzyme that catalyses the decarboxylation of glutamate to γ-amino butyric acid (GABA) and therefore increases the amount of the inhibitory neurotransmitter GABA. Another gene associated with inhibitory neurotransmission, GABA-A receptor subunit alpha 1 (GABRA1), was elevated as well, although not significantly. No differences were seen in glutamate receptor NMDA (N-methyl-d-aspartic acid) subunit 1 (GRIN1, excitatory neurotransmission) and a member of RAB family of small GTPase (RAB3A, regulation of neurotransmitter release). In conclusion, only gene expression for inhibitory neurotransmission was affected in the ipsilateral PFL, which could thus lead to increased inhibition. It is important to point out that the acoustic noise trauma was performed by a 10-kHz 124-dB sound for 1 h. Sounds of this loudness have been proven to induce tinnitus successfully (Bauer and Brozoski, 2001; Von Der Behrens, 2014). Therefore, is probable that some guinea pigs not only suffered from hearing loss, but also from tinnitus.

It was shown that the SFR was increased in the contralateral IC of guinea pigs after unilateral noise trauma in comparison with healthy controls (Vogler et al., 2016). Directly after ablation of the ipsilateral PFL, the SFR in the IC increased even more, but this effect was not present in animals without hearing loss. This study indicates that the PFL has a tonic inhibitory effect on the contralateral IC in animals with hearing loss, but not in animals without hearing loss. In this study, hearing loss was induced by a 2-h exposure to a 10-kHz 124-dB sound, without a tinnitus evaluation. So, also in this study, tinnitus in addition to hearing loss cannot be ruled out.

The dorsal cochlear nucleus (DCN) and the vestibulocerebellum of rats have shown to express the protein doublecortin (DCX) (Manohar et al., 2012). DCX is a protein that is critical for neuronal migration and the development of cerebral cortex, and it has been correlated with neurogenesis (Francis et al., 1999; Von Bohlen Und Halbach, 2011). Therefore, it is used as a marker of neuroplasticity. DCX is found in the UBCs of said regions. UBCs are small, highly specialized, glutamatergic neurons located in the cerebellar cortex and the granule cell domain of the cochlear nucleus, that both receive input from mossy fibres and form synapses with granule cells and other UBCs (Fig. 2). UBCs are located in both the FL and PFL and an unusually high density of UBCs is located in the transition zone between the ventral PFL and the FL (Jaarsma et al., 1998; Manohar et al., 2012; Mugnaini et al., 2011). Freemyer et al. (2019) evaluated DCX staining in the DCN, hippocampal dentate gyrus and PFL in rats 25–30 days after noise exposure. Rats were exposed to a 1-h 16-kHz 114-dB sound unilaterally. No differences were present in DCX staining in the DCN between noise exposed rats and controls. DCX immunoreactivity (IR) in the UBC rich transition zone between the FL and ventral PFL was bilaterally increased in sound damaged animals. These results indicate that hearing loss due to noise exposure induces neuroplasticity in the UBCs of the transition zone of the FL and ventral PFL. Gap detection was used to assess tinnitus-like behaviour, but these results were not robust enough to relate DCX IR to tinnitus-like behaviour. However, it is probable that at least some animals suffered from tinnitus.

In conclusion, following hearing loss (and perhaps tinnitus) due to cochlear trauma, the ipsilateral PFL shows increased inhibition as evidenced by increased gene expression, and neuroplasticity of UBCs at the transition zone of the FL and ventral PFL.

5.2. Tinnitus

Tinnitus is a common auditory condition. It is present in approximately 5.1–42.7% of the general population (McCormack et al., 2016). 3.0–30.9% report their tinnitus to be bothersome and it negatively affects quality of life in 1–4% of the general population (Eggermont and Roberts, 2004; McCormack et al., 2016). Cochlear damage due to noise exposure is the major cause of the onset of tinnitus (Agrawal et al., 2009, 2008). Since tinnitus can still persist after excising the auditory nerve (Berliner et al., 1992), it is thought that tinnitus is due to a maladaptive neuroplastic central response to sensory deprivation (Baguley et al., 2013). Tinnitus is associated with aberrant neuronal (spontaneous) firing because of increased SFR or increased neural synchrony. This may be caused by, for instance, neuroplasticity, changes in inhibitory neurotransmission (f.i. GABA) or tonotopic map reorganisation (Baguley et al., 2013; Eggermont and Roberts, 2004; Knipper et al., 2013; Wang et al., 2011).

The last two decades, emerging data suggest that the PFL is associated with tinnitus as well. Brozoski et al. (2007a) were the first to describe the PFL in the context of tinnitus. They evaluated the distribution of central neural activity in a rat model of tinnitus using manganese-enhanced magnetic resonance imaging (MEMRI). Manganese (Mn^{2+}) is an activity-dependent paramagnetic contrast agent which accumulates in active neurons through voltage gated calcium channels (Silva et al., 2004). Therefore, MEMRI is a remarkably useful method to study tinnitus and brain function (Cacace et al., 2014; Malheiros et al., 2015). In this study, tinnitus was induced by monaural noise trauma. Animals with behavioural evidence of tinnitus showed significantly increased activity in the PFL and posterior ventral cochlear nucleus (PVCN) ipsilateral to the trauma ear, and in the contralateral IC compared to controls without tinnitus (Brozoski et al., 2007a). Normal rats exposed to an artificial tinnitus sound showed increased activation in the ipsilateral PVCN and bilateral DCN, but not in the PFL (Brozoski et al., 2007a). From the MEMRI measurements, it is not clear whether the increased activity resembles increased inhibition or increased excitation and therefore the net effect of the PFL as a whole is not known. Ablation of the PFL presumably reduces inhibitory actions of Purkinje cells on the IC based on the observation that the SFR in the contralateral IC increases after PFL ablation (Vogler et al., 2016). Moreover, because the inhibitory Purkinje cells are the only cerebellar cortical output neurons, it is probable that the increased activity resembles an inhibitory PFL output. When the GABA-agonist Vigabatrin, a drug shown to be effective in eliminating tinnitus in rats (Brozoski et al., 2007b), was systemically administered to tinnitus subjects, no difference in neural activity was present in the auditory nerve, AVCN, PVCN, DCN, IC and PFL between treated tinnitus subjects and controls without tinnitus. Treated tinnitus subjects also showed significantly less activity in the ipsilateral PFL than untreated subjects with tinnitus; i.e. the GABA agonist inhibited the PFL.

In subsequent work of this research group, it is hypothesized that the glutamatergic UBCs in the ventral PFL and DCN may be involved in tinnitus. Using immunohistochemistry with antibodies against DCX, Bauer et al. (2013b) showed that the percent area positively stained for DCX was bilaterally elevated in both the DCN and ventral PFL of rats with tinnitus after unilateral noise exposure compared to controls, with the highest IR in the ipsilateral ventral PFL (Bauer et al., 2013b). The IR in the ventral PFL is in accordance with the results of Freemyer et al. (2019), which showed bilaterally increased DCX IR in the UBC rich transition zone between the FL and ventral PFL in unilaterally sound damaged animals. DCX IR increase cannot discriminate between increased DCX within a cell or an increase in the number of cells expressing DCX. In later studies, a rostro-caudal DCX IR gradient in the DCN, but not in the FL and PFL was demonstrated (Brozoski et al., 2017). This implies that non-systematically sampling within the DCN, f.i. only in de DCX rich part of the DCN, can distort the results. Therefore, the DCX IR in the DCN actually may not be elevated. Nevertheless, the increase of DCX IR in the ventral PFL of rats with tinnitus may indicate that neuroplastic changes in the PFL, and perhaps also the DCN, are associated with tinnitus.

If UBCs indeed are involved in tinnitus, it should be possible to modulate tinnitus using local glutamatergic blockade or activation in the PFL since UBCs are glutamatergic interneurons. Ipsilateral to the sound damaged ear, activation of the PFL glutamatergic receptors (NMDA and AMPA) led to exacerbation of tinnitus in rats with weak pre-drug evidence of tinnitus and to the onset of tinnitus in rats without pre-existent tinnitus (Bauer et al., 2013b). Blocking these receptors by an antagonist cocktail in the ipsilateral PFL attenuated tinnitus partially during treatment in rats with pre-existent tinnitus (Bauer et al., 2013b). Neural activity of the PFL and DCN also significantly decreased in rats treated with a glutamatergic antagonist (D-AP5) by PFL injection (Brozoski et al., 2013). Recapitulatory, it is possible to modulate tinnitus by both glutamatergic blockade and activation in the ipsilateral PFL. As glutamatergic activation resulted in exacerbated tinnitus, these results suggest that noise exposure leads to an upregulation of UBCs and therefore (via Purkinje cells) an increased inhibitory effect of the PFL (Fig. 5A). This is also in accordance with the observed increased gene expression of GAD1, the catalysator of glutamate to GABA, in the ipsilateral PFL after noise exposure as described earlier; it could be the result of upregulation of the UBCs and therefore increased excitation of the inhibitory Purkinje cells (Mulders et al., 2014).

Temporary inactivation of the ipsilateral PFL by lidocaine infusion in the subarcuate fossa resulted in reversable elimination of tinnitus (Bauer et al., 2013a). Ablation of the ipsilateral PFL after tinnitus induction resulted in complete elimination of tinnitus. However, ablation of the PFL before tinnitus induction did not prevent the onset of tinnitus, although it did result in attenuated subsequent tinnitus. General psychophysical performance of rats was not permanently affected by PFL ablation. Some rats showed vestibular symptoms, but these resolved within three days. These results show that once tinnitus is established, the PFL is critical to maintain tinnitus. However, the PFL is not obligatory for onset of tinnitus.

Many of the studies are performed by the same research group. However, few others also studied the role of the PFL in tinnitus. Du et al. (2017) studied in rats the effect of salicylate treatment, a method known to induce tinnitus, on SFRs in the PFL. They showed that the SFR of both Granule cells and UBCs was elevated by salicylate treatment. No significant effect was present on the SFR of Basket, Stellate and Golgi cells, but there was a declining tendency. However, because of a small number of neurons and different numbers of units between salicylate and control group measured, these results should be interpreted with caution. Salicylate treatment also increases the extracellular glutamic acid level in the PFL. Chen et al. (2015) showed using fMRI in rats with salicylate-induced tinnitus enhanced spontaneous activity in both paraflocculi. Moreover, they showed an increased functional connectivity between the auditory cortex and both the PFL and cerebellar lobules IV (Chen et al., 2015). LFP amplitudes and MUC discharge rates were increased significantly after salicylate administration (Chen et al., 2017). Typically, the early response (<15ms) was reduced and the late response enhanced. Herein, the early response
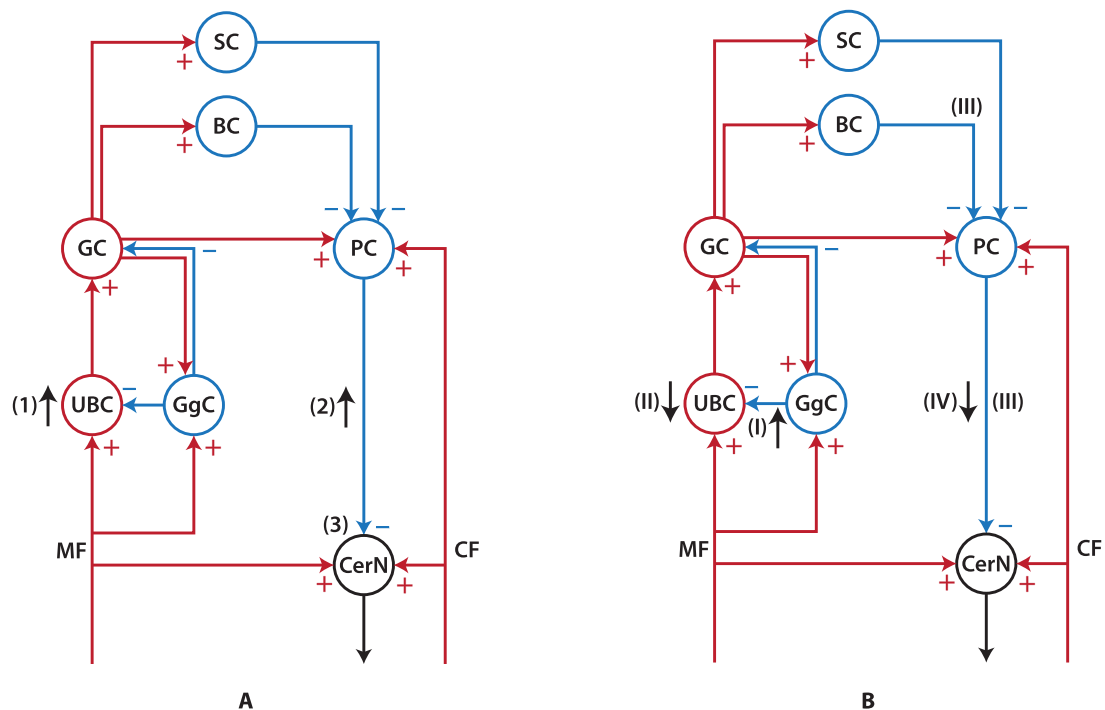
Fig. 5. Activity of paraflocullar neurons. (A) In tinnitus. Cochlear trauma leads to upregulation of UBCs (1). UBCs excite the inhibitory Purkinje cells via Granule cells, leading to increased inhibitory cortical output (2). The inhibitory Purkinje cell output and the excitatory input from mossy fibres and climbing fibres are merged in the cerebellar nuclei (3). (B) In tinnitus treated with a GABA agonist, compared to no treatment. Administration of GABA (I) leads to inhibition of UBCs (II). The Purkinje cells are also more inhibitory, but the reduced inhibition of the Basket cells and Stellate cells approximately evens this out (i.e. disinhibition) (III). However, inhibition of UBCs leads to less excitation of Purkinje cells and therefore a less inhibitory output (IV). Abbreviations: BC, Basket cell; CF, Climbing fibre; CerN, cerebellar nucleus; GC, Granule cell; GgC, Golgi cell; MF, Mossy fibre; PC, Purkinje cell; SC, Stellate cell; UBC, Unipolar Brush cell.

may indicate input from close-by structures such as the cochlea or cochlear nucleus and the late response presumably arises from a multisynaptic pathway (f.i. from the auditory cortex) relaying information to the PFL. The results from these research groups also indicate a role of the PFL in tinnitus.

Summarizing, research suggests that cochlear trauma leads to upregulation of UBCs (Fig. 5A). These UBCs excite the inhibitory Purkinje cells via Granule cells, leading to increased inhibitory PFL output. Tinnitus can be modulated by intervening in the circuitry of the PFL ipsilateral to the trauma ear. Local activation of glutamatergic receptors exacerbated tinnitus, because UBCs and granule cells become more excitatory leading to increased inhibition from Purkinje cells. Tinnitus can be reduced by either administration of GABA (Fig. 5B) or glutamate antagonists, or inactivation of the PFL. Administration of GABA leads to inhibition of UBCs. The Purkinje cells are also more inhibitory, but the increased inhibition of the basket cells and stellate cells approximately evens this out (i.e. disinhibition). However, increased inhibition of UBCs by Golgi cells leads to a less inhibitory PFL output and tinnitus is reduced or completely eliminated. Inactivation or ablation of the PFL removes all PFL inhibitory output and tinnitus is eliminated.

5.3. Distinguishing hearing loss and tinnitus

It is hard to differentiate between the effects of hearing loss and tinnitus. As a result, it is almost impossible to determine when either of both arise. It has been hypothesized that UBCs play a role in tinnitus, perhaps elicited by neuroplasticity. In addition to DCX, UBCs are also immunostained by epidermal growth factor receptor substrate 8 (Eps8). Eps8 mediates the cell's response to epidermal growth factor, has a role in actin polymerization and facilitates cytoskeleton protein-protein interactions. Although the exact function is not yet known, Brozoski et al. (2017) hypothesized that Eps8

elevation could mark cells engaged in plastic dendritic remodelling to improve signal transmission from mossy fibres to granule cells. In this study, hearing loss, and also tinnitus in some animals, was induced by unilateral noise exposure with a peak level of 120 dB, centred at 16 kHz for the duration of 1 h. This exposure procedure resulted in tinnitus in some animals, and lack thereof in others, where tinnitus was assessed by an operant conditioned-suppression procedure. Rats with tinnitus showed an UBC-localized Eps8 IR elevation only in their PFL. No difference was present between the ipsilateral and contralateral PFL. In exposed animals without tinnitus Eps8 and DCX IR was decreased in their FL bilaterally. Contrary to their earlier results which showed elevated DCX IR in rats with tinnitus as described in Section 5.2 (Bauer et al., 2013b), there was no difference in DCX IR in these rats with tinnitus. These results suggest tinnitus-related UBC upregulation or remodelling of synaptic function in the PFL that is not present in animals with hearing loss but without tinnitus.

6. Paraflocculus-auditory cortex feedback loop and tinnitus

Assembling the discussed results leads to the following hypothesis on a feedback-loop of the PFL with the auditory cortex and the role of the PFL in tinnitus (Fig. 6). As described earlier, the PFL presumably receives its main auditory input from the contralateral auditory cortex via the pontine nuclei and possibly some from the ipsilateral IC. Electrical stimulation of the PFL modulated the activity of the contralateral auditory cortex, and ablation of the PFL after noise trauma revealed a tonic inhibitory effect of the PFL on the contralateral IC after noise exposure, so an auditory pathway from the PFL to the contralateral IC and auditory cortex should exist. Since no anatomical efferent auditory connections of the PFL are known, the specific pathway remains to be elucidated. Let us speculate. Both divisions of the PFL project to the dentate nu-
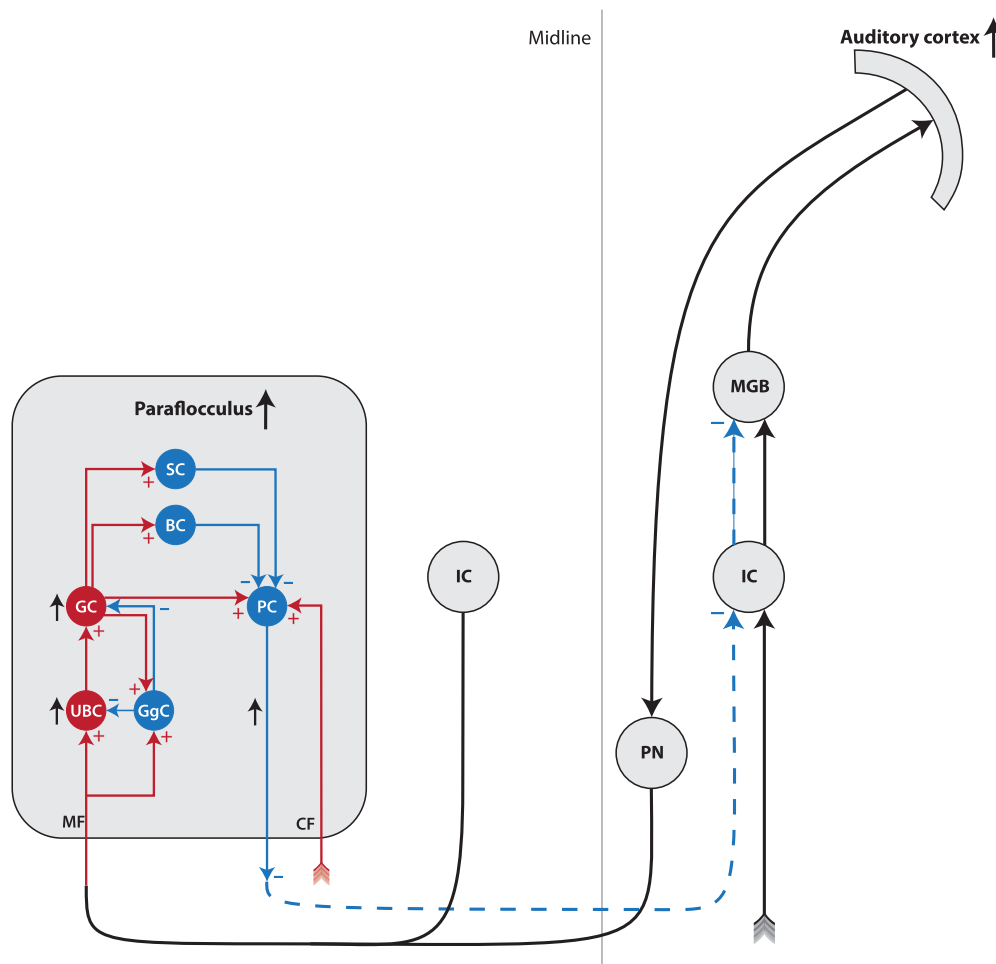
Fig. 6. Proposed paraflocculus-auditory cortex feedback loop and its contribution to tinnitus. Dashed lines resemble presumed connections of which the specific pathway is not known. The PFL receives its auditory input from the auditory cortex via the pontine nuclei. It processes the input and sent its output presumably to the IC and auditory cortex. In tinnitus PFL UBCs are upregulated, which causes an increased inhibitory output of the PFL to the IC via a currently unknown pathway.

cleus, and the lateral and posterior interposed nucleus (Gayer and Faull, 1988; Haines and Whitworth, 1978). Since the dentate nucleus has been shown to respond to auditory stimuli (Wang et al., 1991; Woody et al., 1998; Xi et al., 1994) and the interposed nuclei have not, it is more probable that the dentate nucleus is involved in the PFL auditory pathway. An auditory pathway involving the dentate nucleus was proposed by Wang et al. (1991). This pathway runs between dorsal and ventral cochlear nucleus, dentate nucleus, rostral thalamus and the motor cortex. However, an efferent connection between the dentate nucleus and structures of the classical auditory pathway including the auditory cortex has not yet been described. In tinnitus animals, the neural activity of the DCN was decreased after injection of a glutamatergic antagonist in the PFL (Brozoski et al., 2013). This may suggest a pathway between the PFL and the DCN, which has a connection with the IC and auditory cortex via the classical auditory pathway. In summary, we propose that an auditory feedback loop exists between the auditory cortex and the PFL. The descending part of the loop runs from the auditory cortex via the pontine nuclei to the PFL. The ascending part of the PFL-auditory cortex feedback loop may run from the PFL, perhaps via the dentate nucleus and the DCN, to the IC and auditory cortex.

The question remains what the role of this feedback loop could be in tinnitus and the contribution of the PFL herein. In animals with behavioural evidence of tinnitus, increased connectivity between the auditory cortex and PFL is present (Chen et al., 2015),

which could mean that the PFL-auditory cortex feedback loop becomes more activated. Given the fact that PFL ablation diminished existing tinnitus, but did not prevent the onset of tinnitus, the PFL presumably is a necessary component for maintaining and modulating tinnitus, but is not the (only) generator (Bauer et al., 2013a). Cochlear trauma leads to upregulation of PFL UBCs and therefore an increased inhibitory output from the PFL. After noise exposure, the PFL had a tonic inhibitory effect on the IC. Therefore, increased inhibitory PFL output presumably leads to increased inhibitory IC input. Auditory cortex activity is known to be increased in tinnitus subjects (Eggermont, 2017). The only way inhibitory input in the IC could lead to increased activity in the auditory cortex, is by means of disinhibition due to another inhibitory pathway between the IC and auditory cortex. Twenty to forty percent of neurons between the IC and Medial Geniculate Body are inhibitory and a GABA decrease, and potential loss of GABA mediated inhibition, was present in the contralateral Medial Geniculate Body of noise exposed animals (Beebe et al., 2018; Brozoski et al., 2012). The inhibitory connection between the IC and MGB could be reduced because of the inhibitory effect of the PFL on the IC and therefore lead to an increased auditory cortex activity. This would result in a reduced connectivity between the contralateral IC and auditory cortex, which was shown by Lanting et al. (2014) and Boyen et al. (2014), and highly correlated activity patterns of the thalamic nuclei and auditory cortex as shown by Boyen et al. (2014).

7. Evidence for involvement of the (para)flocculus in human tinnitus

Hardly any study on the auditory function of the PFL makes the distinction between the ventral and dorsal PFL and no neuroanatomical tracing studies with regard to auditory function of the FL, acPFL or tonsil have been performed in humans. Therefore, it is not known whether the human acPFL could be the homologue of the observed PFL auditory and tinnitus effects, or the tonsil. Only one observational study has been performed in humans with regard to the function of the FL or acPFL in tinnitus (Mennink et al., 2018). This study correlated the volume of the FL/acPFL on MRI-scans with the Tinnitus Functional Index (TFI) score, which is an indication of the severity and nuisance of tinnitus. There was a positive correlation between TFI-score and FL/acPFL-complex volume. People with more severe tinnitus had a bigger FL/acPFL-complex and vice versa. A limitation of this study is that it was not possible to delimit the acPFL on the available MRI-scans individually, so the volumes of the FL and acPFL were probably merged. FL and acPFL sizes differ greatly between individuals, with variability of the acPFL being more than double the size of the FL. Size ratios range from 40:1 to 1:1 (Tagliavini and Pietrini, 1984). Therefore, it is conceivable that the acPFL accounts for the biggest part of the variability of the FL/acPFL-complex volume. Assuming that volume is related to function, it could be the case that a smaller acPFL corresponds to fewer numbers of upregulated UBCs and therefore less inhibitory output via the Purkinje cells. This could explain the positive correlation between FL/acPFL-complex volume and TFI-score and points to the acPFL as having a role in tinnitus in humans. At present, this explanation remains speculative and requires to be studied more thoroughly. Moreover, it could be possible that FL/acPFL-complex volume is also related to hearing loss severity, which should be elucidated in future studies.

7.1. Gaze-modulated tinnitus

A special type of tinnitus in humans is gaze-evoked or gaze-modulated tinnitus (GMT), in which perceptual characteristics are modulated by a horizontal or vertical eye gaze deviation from a neutral head position. It is almost exclusively described after removal of a vestibular schwannoma by cerebellopontine angle surgery, with a prevalence ranging from 19% to 51% (Baguley et al., 2006; Biggs and Ramsden, 2002; Mennink et al., 2018). However, surgical removal of any space-occupying lesion affecting the vestibulocochlear nerve can lead to GMT (Coad et al., 2001).

Several surgical methods for removal of a cerebellopontine angle tumour exist, with the retrosigmoid (RS) and translabyrinthine (TL) approach as the most common. Interestingly, the prevalence of GMT differs between these approaches with 19–36% for TL and 58% for RS (Baguley et al., 2006; Biggs and Ramsden, 2002; Mennink et al., 2018). This difference might be explained by the amount of FL and/or acPFL manipulation by the surgeon or compression by the tumour. In the TL approach, the tumour is surgically assessed via the auditory canal and vestibulum auris. Small tumours reside mainly in the internal auditory canal with little or no protrusion in the cistern. Large tumours expand into the cistern and may give rise to compression of cerebellar structures. The amount of FL and/or acPFL manipulation during TL surgery is therefore dependent on tumour volume, with no or minimal manipulation in small tumours and more manipulation in larger tumours. In the RS approach, the tumour is assessed via a route posterior to the sigmoid sinus. It leaves the vestibulum auris and cochlea intact, but it nearly always requires manipulation of the FL and the acPFL to be able to access the tumour. So, the amount of surgical manipulation differs between both surgical approaches,

which could explain the differences in prevalence of GMT. Also, patients with a smaller FL/acPFL-complex suffered from GMT more often. This suggests that atrophy due to the surgery or the tumour impaired the function of the Fl/acPFL, which may have caused GMT (Mennink et al., 2018).

The FL and ventral PFL monitor and adjust eye movements in animals (Fukushima, 2003; Voogd and Wylie, 2004). De Zeeuw et al. (1994) studied FL projections in rabbits. The rabbit FL can be divided in zones with each its own function. Neurons in zone 1 and 3 respond best to eye rotation around a horizontal axis and in zone 2 and 4 best to rotation in a vertical axis. A similar distribution of these response properties is present in monkeys, measured in the FL and the ventral PFL (Krauzlis and Lisberger, 1996). A majority of humans experience GMT only in horizontal eye gaze directions (Baguley et al., 2006), thus corresponding to zone 1 and 3 in the rabbit FL. These zones of the FL project to the ventral dentate nucleus, ventral and dorsal group y, and the superior vestibular nucleus (de Zeeuw et al., 1994; Voogd and Wylie, 2004). The ventral PFL shares similar projections, with the addition of the posterior interposed and dentate nuclei (Dietrichs, 1981; Nagao et al., 1997). Group y are lateral extensions of the superior vestibular nucleus that gives rise to projections to the oculomotor nuclei. Dorsal group y also projects to the part of the inferior olive that projects to the corresponding FL zone, therefore providing a closed pathway. So, not only do FL and ventral PFL respond to eye movements, they also have a closed pathway with the inferior olive, cerebellar nuclei, and the part of the vestibular nuclei that projects to oculomotor nuclei (de Zeeuw et al., 1994). In line with these pathways, it was shown that patients with large cerebellopontine angle tumours, extensive oculomotor abnormalities occur, with gaze nystagmus as the most common one, due to compression of the FL and acPFL (Nedzelski, 1983).

The exact mechanism in which the FL and/or the acPFL would modulate tinnitus because of eye gaze remains unknown. The diversity in used terminology for the FL and PFL in papers complicates drawing conclusions on which structure is responsible for tinnitus and GMT in humans. In non-human mammals, mainly the PFL has been implicated in tinnitus, which points to the human acPFL and/or cerebellar tonsil as having a role in tinnitus. Because the acPFL is manipulated during cerebellopontine angle surgery and the cerebellar tonsil is not or minimally, is seems the most logical to appoint the GMT effect to the acPFL.

8. Conclusions

Although the FL and PFL are strongly related to the vestibular system, animal studies indicate that the FL also receives auditory input from the cochlea and the cochlear nucleus, and that the PFL indirectly receives auditory input from the auditory cortex. Efferent auditory projections of the FL and PFL have not yet been described, but the ability of the PFL to modulate neural activity of the auditory cortex suggests their existence. We propose that an auditory feedback loop exists between the auditory cortex and PFL. Animal studies suggest the PFL as an important component in the mechanism of tinnitus. It can modulate tinnitus and when ablated, the tinnitus is diminished. Extrapolating these results to humans provides a challenge. The non-human mammalian ventral PFL and dorsal PFL are homologues of the human acPFL and cerebellar tonsil, respectively. Since hardly any animal study on tinnitus distinguished the ventral and dorsal PFL, it is not known whether the acPFL or the cerebellar tonsil is also responsible for tinnitus modulation in humans. The few studies performed in humans point to the acPFL. However, more research is needed to clarify the role of the acPFL in tinnitus. As the (para)flocculus is accessible via neurosurgical procedures, it may be a suitable target for treatments such as deep brain stimulation, ablation or local pharmaceutical inter-

vention. This warrants further efforts into understanding the role of the (para)flocculus in tinnitus.

Declaration of Competing Interest

None.

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