

## REVIEW

# Review of salicylate-induced hearing loss, neurotoxicity, tinnitus and neuropathophysiology

## *Effetti clinici e neurofisiopatologici del salicilato: ipoacusia, acufene e neurotossicità*

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## SUMMARY

Salicylate's ototoxic properties have been well established, inducing tinnitus and a sensory hearing loss when administered in high doses. Peripherally, acute dosing of salicylate causes frequency dependent reductions in DPOAEs and CAP amplitudes in low (<10 kHz) and high (>20 kHz) frequencies more than mid frequencies (10-20 kHz), which interestingly corresponds to the pitch of behaviourally-matched salicylate-induced tinnitus. Chronic salicylate dosing affects the peripheral system by causing a compensatory temporary enhancement in DPOAE amplitudes and up-regulation of prestin mRNA and protein expression. Despite salicylate's antioxidant properties, cultured cochlea studies indicate it also impairs spiral ganglion neurons (SGNs) by paradoxically causing an upsurge of superoxide radicals leading to apoptosis. Centrally, salicylate alters  $\gamma$ -aminobutyric acid (GABA) and serotonin mediated neurotransmission in the central nervous system (CNS), which results in classical and non-classical auditory regions showing hyperactivity after salicylate administration. In the auditory cortex (AC) and lateral amygdala (LA), neuron characteristic frequencies (CF) shift upward and downward to mid frequencies (10-20 kHz) altering tonotopy following salicylate administration. Additionally, current source density (CSD) analysis showed enhanced current flow into the supergranular layer of the auditory cortex after a high systemic dose of salicylate. In humans, auditory perception changes following salicylate or aspirin, including decreased word discrimination and temporal integration ability. The results of previous studies have partially identified the mechanisms that are involved in salicylate-induced tinnitus and hearing loss, however to date some interactions remain convoluted. This review discusses current knowledge of salicylate ototoxicity and interactions.

**KEY WORDS:** Sodium salicylate (SS) • Distortion product otoacoustic emissions (DPOAE) • Compound action potential (CAP) • Inferior colliculus (IC) • Auditory cortex (AC) • Lateral amygdala (LA) • Characteristic frequency (CF) • Current source density (CSD)

## RIASSUNTO

*Gli effetti ototossici del salicilato sono ben noti ed includono, ad alti dosaggi, acufene ed ipoacusia transitoria. In periferia, la somministrazione acuta di salicilato nell'animale induce una riduzione d'ampiezza dei prodotti di distorsione (DPOAE) e dei potenziali d'azione (CAP), prevalentemente per le basse (<10 kHz) e per le alte (>20 kHz) frequenze; è interessante come questa alterazione corrisponda alla tonalità dell'acufene indotto sperimentalmente. La somministrazione cronica causa invece un aumento transitorio dell'ampiezza dei DPOAEs ed una up-regulation dell'mRNA e dell'espressione proteica della prestina. In vitro la tossicità da salicilato si evidenzia prevalentemente a livello dei neuroni del ganglio spirale (SGNs) inducendo, a dispetto delle ben note proprietà antiossidanti, un rilascio paradossale di radicale superossido che avvia la catena apoptotica. Centralmente, il salicilato altera la trasmissione GABA e serotonino-mediata inducendo una iperattività di specifiche popolazioni neuronali. A livello della corteccia uditiva (AC) e dell'amigdala laterale (LA) le frequenze caratteristiche neuronali (CF) variano alterando la tonotopia fisiologica, specialmente per le frequenze centrali (10-20 kHz). Inoltre, l'analisi della densità di corrente (CSD) ha dimostrato un maggior influsso negli strati supragranulari della corteccia uditiva in seguito alla somministrazione di dosi elevate di salicilato per via sistemica.*

*Nell'uomo gli effetti ototossici del salicilato, oltre ad ipoacusia transitoria ed acufene, includono una diminuita discriminazione verbale e difficoltà nell'integrazione temporale. Sebbene diversi lavori in letteratura abbiano identificato i meccanismi fisiopatologici alla base delle alterazioni uditive indotte dal salicilato, ad oggi alcune interazioni rimangono poco chiare.*

**PAROLE CHIAVE:** Sodio salicilato (SS) • Prodotti di distorsione delle otoemissioni acustiche (DPOAE) • Potenziali d'azione composti (CAP) • Collicolo inferiore (IC) • Corteccia uditiva (AC) • Amigdala laterale (LA) • Frequenze caratteristiche (CF) • Densità di corrente (CSD)

## Introduction

The active ingredient in aspirin, salicylate, is a commonly used antipyretic, analgesic and anti-inflammatory drug. However, consumption in large doses (6-8 gm/day)<sup>1</sup> is widely known to induce hearing loss and tinnitus<sup>2,4</sup>. Originally, the influence of salicylate on the auditory system was thought to be temporary, but more recent discoveries show that prolonged, high doses of sodium salicylate (SS) can cause sustained damage within the inner ear, suppressing the neural output of the peripheral system<sup>5</sup>. In addition, despite salicylate's antioxidant properties, high doses *in vitro* cause a paradoxical up-regulation of the superoxide radical, leading to apoptosis in spiral ganglion neurons (SGN)<sup>6</sup>. Unbound salicylate concentrations in plasma have a high correlation with the severity of induced hearing loss<sup>2,7</sup>, which saturates at ~40 dB; however, the level of salicylate in serum is somewhat less predicative<sup>8,9</sup>. Since high doses of salicylate can reliably induce hearing loss and tinnitus, it is commonly used to study its behavioural, anatomical, physiological and perceptual effects on the auditory system<sup>2,10,11</sup>. Peripherally, salicylate influences hearing sensitivity in low (<10 kHz) and high (>20 kHz) frequencies more than mid frequencies (10-20 kHz)<sup>5</sup>. After a high dose of salicylate, neurons in the central auditory system tuned to low characteristic frequencies (CF) in the auditory cortex (AC) shift upward and neurons tuned to very high frequencies shift downward resulting in an over representation at the mid frequencies<sup>12</sup>. In contrast to the suppressed neuronal output peripherally following systemic administration of SS, the AC becomes hyperactive at high levels of auditory stimulation<sup>13-15</sup>. Alterations to the AC may be due to salicylate's influence on  $\gamma$ -aminobutyric acid (GABA) and serotonin mediated neurotransmission in the central nervous system (CNS)<sup>14,16-18</sup>. Since salicylate is known to alter neurotransmitters located throughout the CNS, non-classical auditory structures that also respond to sound, like the amygdala, have also shown neuronal hyperactivity and CF tuning shifts following systemic administration of salicylate<sup>19</sup>.

Despite reliable salicylate-induced threshold shifts and tinnitus generation in animal models, objective and subjective perceptual alterations in humans appear to be more variable<sup>2,20-22</sup>. Over the past several years knowledge about the effects that salicylate imposes on the peripheral and central auditory system has increased significantly. In this review, we will highlight several of the important changes we have identified in the cochlea and central nervous system after administering high doses of salicylate sufficient to induce tinnitus and hearing loss. In addition, we will review some *in vitro* and *in vivo* data from our lab that highlights the neurotoxic effects of salicylate.

## Peripheral effects

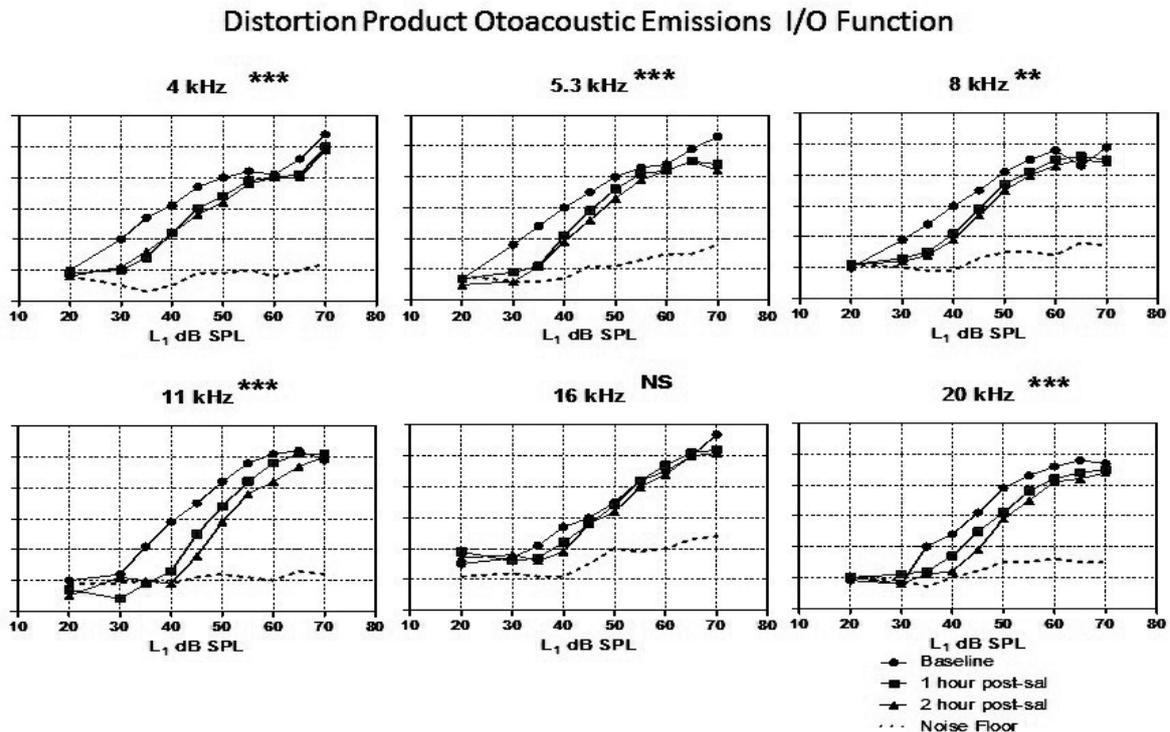
### Distortion Product Otoacoustic Emissions (DPOAE)

#### *Acute effects*

DPOAE is a measurement used to assess the function of the outer hair cells (OHC) in the cochlea. The distortion product is generated by a combination of the electromotile response of OHCs mediated by the motor protein prestin and the +80 mV endocochlear potential<sup>23,24</sup>. The motor protein prestin is part of a family of antiporters that transfer anionic molecules across cell membranes<sup>25</sup>. Prestin, which lines the lateral wall of the OHC, changes shape in response to fluctuations in the voltage across the OHC membrane. OHC depolarisation causes an axial shortening of the OHC whereas hyperpolarisation causes OHCs to elongate<sup>26</sup>. Sound waves lead to motion of the basilar membrane, depolarisation and hyperpolarisation of OHCs causes the axial motion of the OHCs which results in a frequency-dependent amplification of the basilar membrane in response to the incoming sound<sup>26</sup>. Salicylate affects the OHC electromotility response by displacing chloride and binding to the anion-binding sites on prestin, suppressing the amplification properties of the cochlea<sup>23</sup>. Sodium salicylate (SS) causes a frequency-dependent reduction in DPOAE<sup>12</sup>. Figure 1 shows the mean DPOAE input/output (I/O) response of 6 Sprague-Dawley rats under ketamine/xylazine (50/6 mg/kg) anaesthesia. Prior to SS treatment all six frequencies ( $2f_1-f_2=4, 5.3, 8, 11, 16, 20$  kHz) showed robust responses. However, 2 hours post injection (300 mg/kg, i.p.), DPOAEs decreased significantly in the low frequencies ( $2f_1-f_2 < 11$  kHz) and high frequencies ( $2f_1-f_2 > 16$  kHz) but had less influence on the mid frequencies ( $2f_1-f_2$  11 -20 kHz)<sup>12</sup>. While salicylate caused a significant reduction in DPOAEs indicating a sensory hearing loss, animal behavioural models have also indicated that this dose reliably induces tinnitus<sup>3,27-29</sup>. The significant reduction in low and high frequency responses could lead to mid-frequency expansion of the tonotopic map of the AC. Interestingly, animal models have indicated that salicylate induced a mid-frequency perception of tinnitus<sup>30</sup>, consistent with the frequency-dependent reduction in DPOAE<sup>12</sup>.

#### *Chronic effects*

Chronic salicylate treatment also influences the motor protein prestin. Chronic treatment with SS enhanced DPOAE amplitudes and caused an up-regulation in prestin mRNA and protein expression<sup>5,31</sup>. Rats were chronically treated over two time periods consisting of four days, with a two day rebound period in between. During each period, the animals were treated with a systemic injection (300 mg/kg/day) of SS and DPOAEs were measured 2-hours post administration. Changes in DPOAE amplitudes were nor-



**Fig. 1.** Schematic of mean DPOAE amplitudes plotted as a function of  $L_1$  intensity pre- salicylate treatment, 1 h post-salicylate treatment, and 2 h post salicylate treatment (300 mg/kg i.p.). Frequencies (4, 5.3, 8, 11, 16, & 20 kHz) indicated above each panel represent  $2f_1-f_2$ . Acute systemic salicylate administration significantly reduced DPOAE amplitudes in low (4, 5.3, 8, and 11 kHz) and high (20 kHz) frequencies but not at mid (16 kHz) frequencies. (\*\*  $P=0.01$ , \*\*\*  $P=0.001$ , ns=not significant).

malised to pre-treatment amplitudes. During both treatment periods, DPOAE amplitudes were significantly reduced. However, each treatment period was followed by a significant rebound enhancement of DPOAE amplitudes compared to pre-treatment amplitudes<sup>5</sup>. There was no change in DPOAE amplitudes after a long duration treatment with salicylate at moderate levels (200 mg/kg/day, five days a week, for three weeks)<sup>5</sup>.

Interestingly, chronic salicylate treatment increases prestin mRNA expression. Adult guinea pigs received a systemic injection of SS (200-250mg/kg) twice a day for 2 weeks. Prestin mRNA expression progressively increased following daily administrations. Western blots indicated an increase in the prestin protein<sup>31</sup>. Four weeks after cessation of SS treatment prestin mRNA levels returned to normal<sup>31</sup>. These results indicate that as a result of chronic high doses of salicylate, the electromotile function of the OHCs is enhanced, leading to greater cochlear amplification. Some have hypothesised that tinnitus may be generated as a result of an imbalance between IHC and OHC activity<sup>32</sup>. The up-regulation in prestin seen in the OHCs as a result of chronic high dosing of salicylate may cause such an imbalance and contribute to the perception of tinnitus. Indeed, chronic low therapeutic doses of aspirin can cause tinnitus alone without hearing loss<sup>2,33</sup>. However, currently, there are no studies that have indicated an up-regulation of prestin protein as a result of a chronic, low

doses of salicylate. Taken together, these results indicate that chronic high doses of salicylate administration can have long lasting effects on cochlear sensory cells, contributing to sensory hearing loss, tinnitus and possibly plastic changes to the central auditory system.

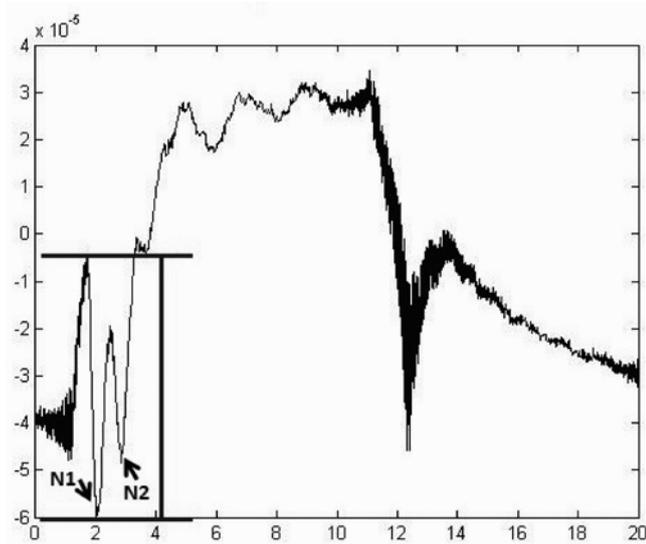
### Compound Action Potential (CAP)

#### Acute effects

The first negative peak (N1) of the electrical response from the round window of the cochlea in response to a click or tone burst is the compound action potential (CAP), which reflects the synchronous onset response of type I auditory nerve fibres that directly connect to inner hair cells (IHC) (Fig. 2). Salicylate is known to depress the cochlear CAP<sup>5,12</sup>. Figure 3 shows changes that occur in the CAP I/O function to tone bursts (4, 12, 16, & 30 kHz) in rats under ketamine/xylazine anaesthesia (50/6 mg/kg, i.p.). Sprague-Dawley rats were treated with SS (300 mg/kg, i.p.) or the equivalent dose of saline, and two hours later the CAP was measured by placing a silver electrode on the round window. The amplification produced by the OHCs results in a non-linear CAP I/O function prior to salicylate treatment (compare to dashed lines in Fig. 3 which represent a linear relationship). The influence of salicylate on the electromotility of OHCs in-

duced a significant threshold shift (I/O functions shifted to the right 20-30 dB), reduced the CAP amplitude and creates a more linear I/O function. The non-linear relationship was affected most at low (4 kHz) and high frequencies (30 kHz) and least at mid frequencies, indicating that cochlear amplification was still largely functional at the mid-frequencies<sup>5</sup>. These results are consistent with

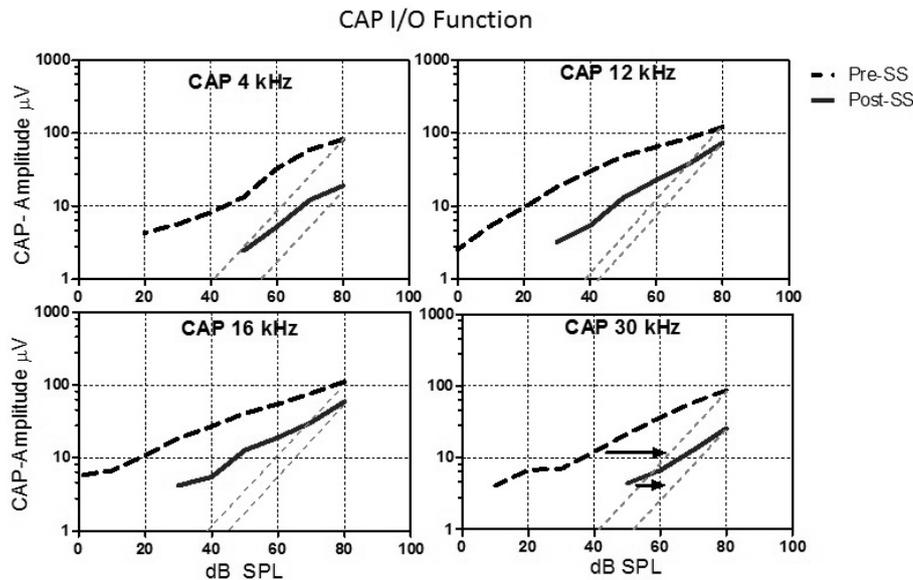
DPOAE data indicating a frequency-dependent reduction in cochlear amplification. Importantly, CAP amplitudes are reduced at high stimulation levels, particularly at 4 and 30 kHz (e.g. amplitude decreased from ~90  $\mu\text{V}$  to ~20  $\mu\text{V}$  at 80 dB SPL), where little cochlear amplification is necessary. This indicates that salicylate also imposes an acute effect on the IHC and/or SGN<sup>6,34</sup>.



**Fig. 2.** High-pass filtered (>11 kHz) CAP response to a 12 kHz tone burst. Bars represent N1; response amplitude represents activity stemming from type 1 auditory nerve fibres. N1 is measured to represent CAP amplitudes.

*Chronic effects*

High doses of aspirin and SS have long been thought to exert only temporary effects on the auditory system; however, recent studies suggest that high doses may induce permanent changes. Most *in vitro* and *in vivo* studies indicate that prolonged treatment with high doses of SS does not damage sensory hair cells *in vitro* or *in vivo*; however, it can affect SGNs<sup>6,34,35</sup>. To determine the effect of chronic salicylate treatment on the CAP, 6 rats were administered 200 mg/kg (i.p.) of SS 5 days a week for 3 consecutive weeks and a control group was given saline<sup>5</sup>. CAP I/O functions were measured four weeks after cessation of treatment. The SS-treated group showed a slight, but significant reduction in CAP amplitudes compared to the control group. CAP I/O functions were still non-linear in both groups indicating normal OHC function. However, when amplitudes were compared as a function of frequency, low and high frequencies were reduced more than mid-frequencies, consistent with the acute effects of SS on OHC functions<sup>5</sup>. Since the CAP was reduced in the chronic treatment group, but sensory cells appeared normal, these results imply that SS induced functional or structural damage to SGN. These results are consistent with auditory brainstem response (ABR) data showing a reduction of ABR amplitudes predominantly at low and high frequencies at high stimulation levels after chronic treatment with high doses of SS<sup>5</sup>.



**Fig. 3.** CAP I/O function pre- (dotted) and 2 hours post-SS (solid) treatment. Dotted grey lines represent linear relationships to the CAP I/O function; the distance between the linear lines and CAP I/O functions represents the amount of residual amplification from the OHCs (demonstrated in 30 kHz I/O panel). At low (4 kHz) and high (40 kHz) frequencies the CAP I/O function becomes more linear than mid frequencies (12 & 16 kHz). The threshold shift is greater in the low and high frequencies (~30 dB SPL) compared to the mid frequencies (~20 dB SPL). CAP I/O functions demonstrate salicylate-induced hearing loss affects the OHC amplification properties in a frequency dependent manner, which is consistent with previous findings in DPOAE I/O functions. CAP amplitudes at high stimulation levels are reduced even though little amplification is needed. This is indicative that salicylate may also affect SGNs.

*Cochlear Microphonic (CM) and Summating Potential (SP)*

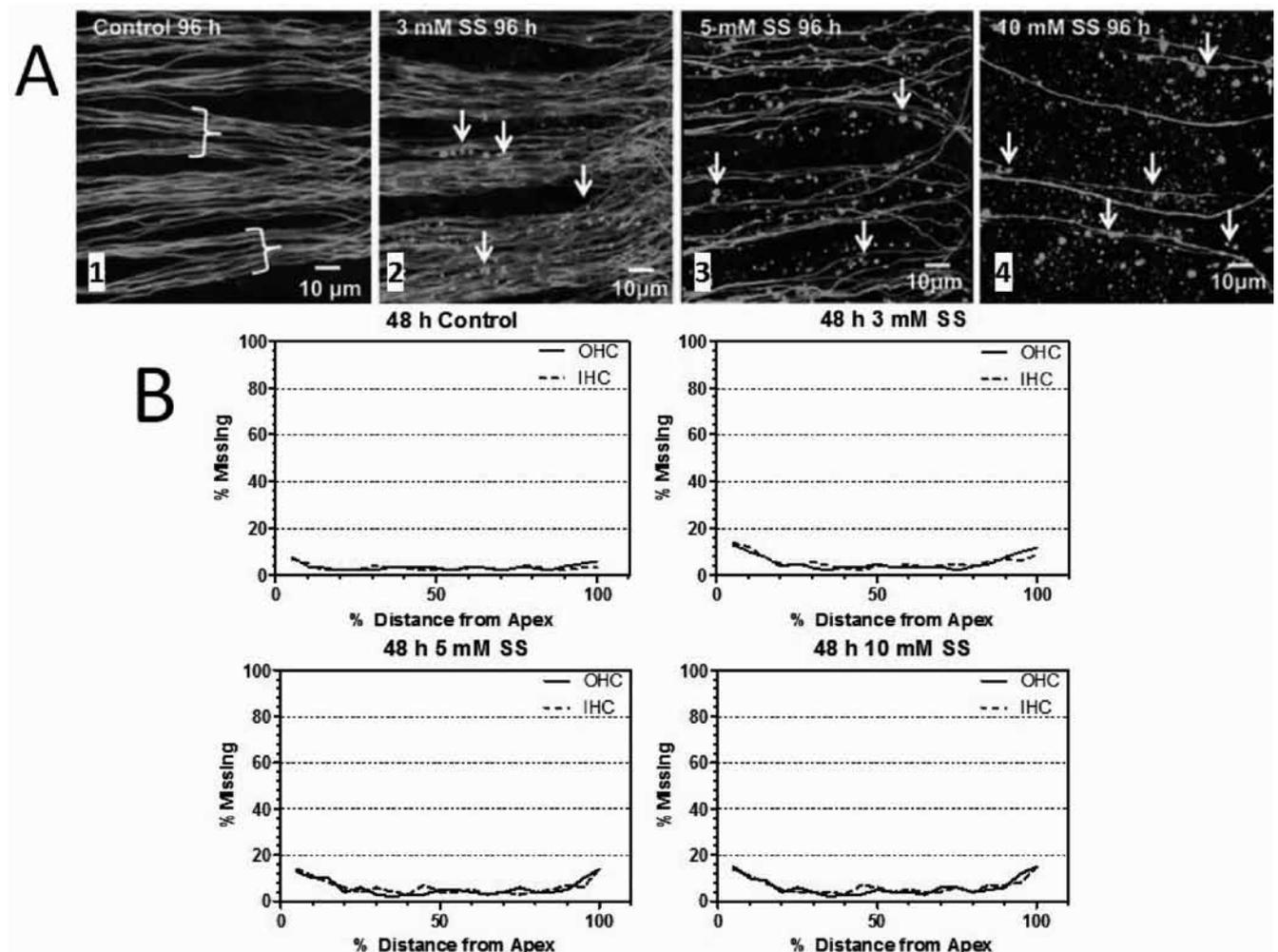
The effects of high doses of SS on the CM and SP have also been studied after systemic or local application of SS. The CM, generated predominantly by the OHCs in cooperation with +80 mV endocochlear potential, largely reflects the flow of potassium ions through the stereocilia on the apical pole of the OHCs in response to acoustic stimulation<sup>36</sup>. The SP, a sustained DC

potential evoked by sound stimulation, is predominantly generated by IHCs along with a smaller contribution from OHCs<sup>37</sup>. The effects of salicylate on the CM and SP in the guinea pig cochlea have been investigated by cochlear perfusions with salicylate followed by recording neural responses to tones<sup>38</sup>. The CM in response to a 10 kHz tone burst was largely unaffected by cochlear perfusion of salicylate<sup>38</sup>; however, others have found an increase in the CM response to a 1 kHz tone<sup>39</sup>. No significant change in the SP was seen following cochlear perfusion in the guinea pig. This functional data suggest that intracochlear perfusion of SS has little effect on hair cells.

#### *Spiral Ganglion Neuron (SGN)*

Recent research has demonstrated that high doses of SS can damage the SGN without concurrent damage to cochlear sensory cells<sup>34,35</sup>. In order to evaluate the effects of

salicylate on the SGN, postnatal day 3 organotypic cultures were treated with SS for 48 hours. Hair cells were labelled with Alexa-488 conjugated phalloidin and auditory nerve fibres were immunolabelled with a monoclonal antibody targeting class III  $\beta$ -tubulin. SS treatment did not induce hair cell loss even at the highest dose of 10 mM; however, the peripheral fibres projecting out from the SGN to the sensory cells were decreased in number and showed many blebs and breaks which were positively correlated with increases in the dose of SS<sup>34</sup>. Figure 4-A shows the peripheral fibers from SGNs cultured under normal conditions (Figure 4-A1) and after being exposed for 96 h to 3 mM (Figure 4-A2), 5 mM (Figure 4-A3) and 10 mM (Figure 4-A4) SS. Nerve fibers exposed to SS showed fragmentation, blebs, and breaks that increased with the concentration of SS. The mean cochleograms in Figure 4-B shows the percentages of missing OHC and IHC in control cultures and cultures treated with 3, 5 or



**Fig. 4.** (A) SGN fibres from the middle turn of the cochlea immunolabelled with primary antibody against  $\beta$ -tubulin and secondary antibody conjugated to Alexa Fluor 488 in control cultures and in 3, 5 and 10 mM SS for 96 hours. In control cultures, nerve fibres demonstrate thick healthy fascicles. With increasing concentrations of SS the nerve fibres show increasingly more fragmentations and blebs (white arrows), and ultimately nerve fiber disappearance in a dose dependent manner. Image obtained from Deng 2013, with permission. (B) Cochleogram representing the percentage of sensory cell loss in control cultures and cultures treated with 3, 5 and 10 mM SS for 48 hours. SGN fibres are degenerated when exposed to SS in a dose dependent manner; however, sensory cells appear structurally unaffected.

10 mM SS. These results indicate that even the highest dose of SS does not destroy hair cells<sup>6</sup>. In addition, recent *in vivo* studies indicate that high doses of SS can lead to SGN degeneration through caspase-mediated apoptosis<sup>40</sup>. Paradoxically, salicylate is a potent antioxidant with neuro- and oto-protective properties<sup>41,42</sup>. However, high doses of salicylate cause an upsurge of the highly toxic superoxide radical in SGNs but not neighbouring sensory and supporting cells<sup>6</sup>. Little or no dihydroethidium (DHE) staining, which labels the superoxide radical, was observed in control cultures. In cochlear cultures treated with 10 mM SS for 48 hours, a significant amount of DHE staining was observed in SGNs, but not in neighbouring sensory or support cells<sup>6</sup>. When cultures were treated with 10 mM SS plus 100  $\mu$ M PyP, a cell permeable superoxide scavenger, they showed significantly less SGN damage than those treated with SS alone<sup>6</sup>. Thus, for reasons yet unknown, high doses of SS exert their toxic effects on SGN by selectively increasing the production of the superoxide radical in SGN, but not other cells in the inner ear.

#### Auditory Nerve (AN)

Auditory nerve fibre recordings following high doses of SS treatment have yielded variable results, which may be a result of the dosage, route of administration or species differences. In cats, a significant increase in spontaneous auditory nerve firing was observed following an extremely high dose of SS (400 mg/kg, *i.v.*)<sup>43</sup>. In contrast, in gerbils, a slight but significant reduction in auditory nerve firing rate following a moderate dose of SS (200 mg/kg *i.p.*) was observed in fibres with low characteristic frequencies (CFs), but not in fibres with high CFs<sup>44</sup>. However, cross comparisons between these species is unreliable due to the cats' inability to effectively metabolise salicylate<sup>45,46</sup>. The effects of chronic treatment of salicylate on spontaneous auditory nerve activity has also been evaluated<sup>47</sup>. The average spectrum of electrophysiological cochleoneural activity (ASECA), a measurement of auditory nerve activity, was recorded from the round window in guinea pigs over several weeks of salicylate administration (200 mg/kg/day, *i.m.*). Initially, the ASECA decreased in the followings hours after salicylate administration; however, after several days this suppression was alleviated and returned to normal levels. Over the following weeks the ASECA progressively increased; furthermore, after cessation of treatment the ASECA reversed and progressively decreased to the values measured initially<sup>47</sup>. The increase in auditory nerve spontaneous activity seen in these studies was suggested as the neural correlate of tinnitus<sup>47,48</sup>; however, the decrease seen in other reports raises questions about this model.

Taken together, the results indicate that salicylate's effect on the peripheral auditory system results primarily in a reduction of auditory sensitivity (threshold shift) caused

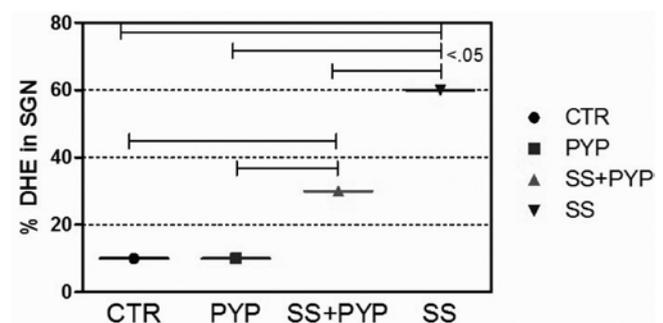
by the frequency-dependent suppression of OHC electromotility. Salicylate's influence on hearing sensitivity was previously thought to be temporary; however, recent data suggest that prolonged treatment with high doses of salicylate may lead to sustained OHC dysfunction<sup>5</sup> and degeneration of SGNs<sup>6,34</sup>. While some studies have reported an increase in spontaneous activity in the auditory nerve after SS treatment others have reported a decrease or no change<sup>44</sup>. Thus, it remains an open question as to what role auditory nerve spontaneous rates play in tinnitus perception, particularly since severe cochlear damage largely abolishes spontaneous activity<sup>49</sup>.

## Central effects

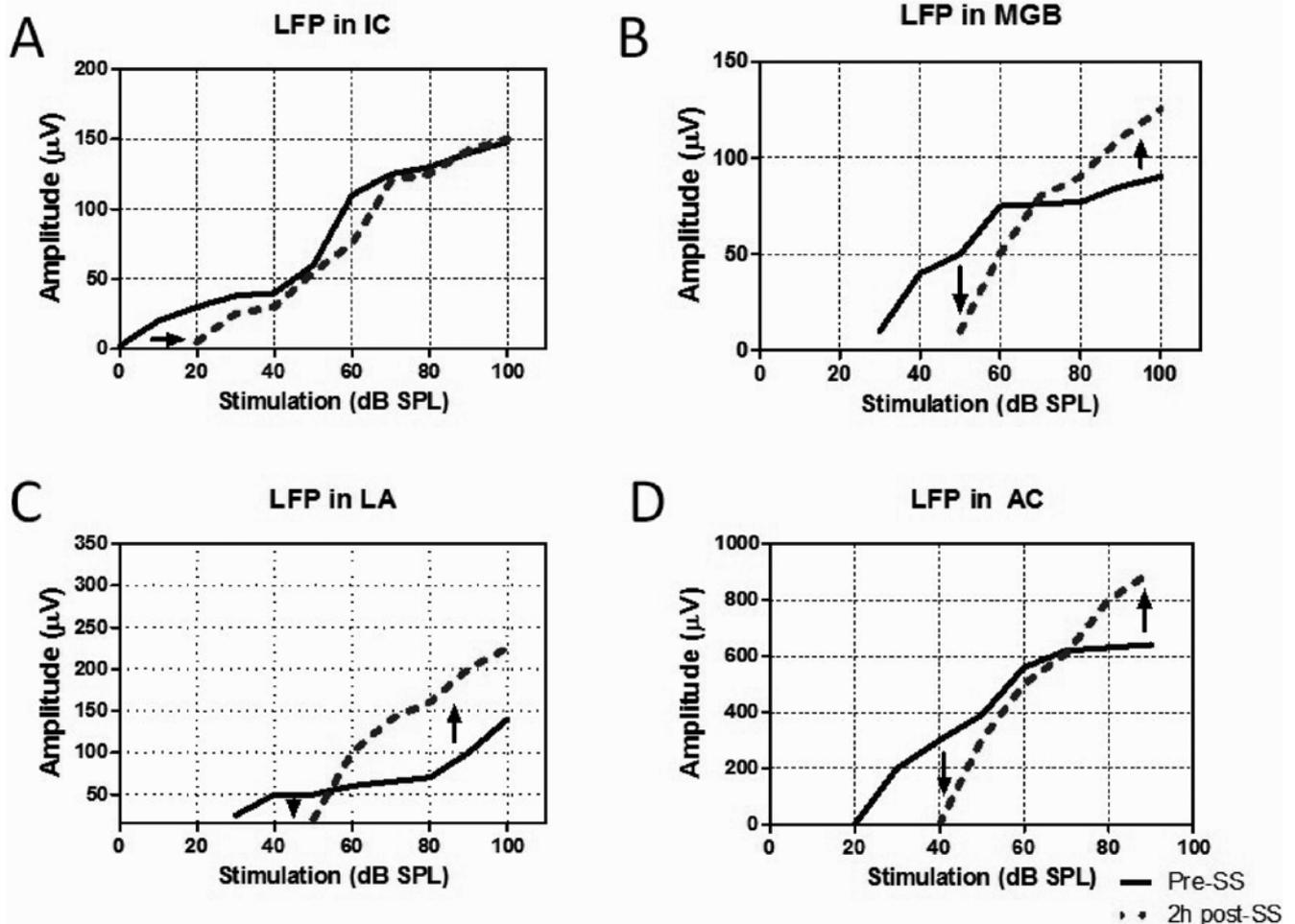
#### Inferior Colliculus (IC)

The IC was one of the earliest auditory brain regions used to investigate salicylate's effects on the central nervous system (CNS). The main inhibitory neurotransmitter in the CNS,  $\gamma$ -aminobutyric-acid (GABA), plays an important role in IC function. GABA-mediated inhibition plays a major role in shaping frequency tuning, binaural processing, and intensity coding in the IC<sup>16,50-52</sup>. Moreover, SS appears to modulate GABAergic activity indirectly by imposing suppressive effects on serotonergic-influenced GABAergic synaptic transmission<sup>18</sup>.

Electrophysiological responses in the IC do not show sound-evoked hyperactivity following salicylate administration unlike higher levels in the central auditory system (Fig. 6-A)<sup>13</sup>. However, because the IC response amplitudes are nearly normal at suprathreshold levels whereas the CAP responses are reduced, these results imply that



**Fig. 5.** The amount of DHE-positive staining in SGNs in control cultures, cultures treated with 100  $\mu$ M PyP alone, 100  $\mu$ M PyP plus 10 mM SS, and 10 mM SS alone. There was no significant difference between the control cultures and cultures treated with PyP alone. The percentage of DHE-positive staining in cultures treated with 100  $\mu$ M PyP plus 10 mM SS was significantly larger than the control or PyP alone cultures ( $P < 0.05$ ). Cultures treated with 10 mM SS alone showed significantly more DHE-positive staining than control, PyP alone and PyP plus SS cultures. This demonstrates that cochlear cultures that were exposed to 10 mM SS alone for 48 hours showed a significant upsurge in superoxide radical. When 100  $\mu$ M PyP was combined with 10 mM SS there was significantly less superoxide present in the cochlea cultures. This indicates that PyP can protect against SS-induced upsurges in superoxide radicals that can lead to SGN apoptosis.



**Fig. 6.** The effects of systemic salicylate on the LFP of the IC, MGB, LA, and AC. (A) LFP in the IC pre- and 2 hours post- systemic administration of SS (250 mg/kg i.p.). Salicylate did not change the amplitudes recorded from the IC; however, an approximately 20 dB SPL threshold shift occurred. The threshold shift is most likely due to salicylates suppressive effects on OHC electromotive amplification. (B) LFP in the MGB pre- and 2 hour post- systemic administration of SS (300 mg/kg i.p.). There was an approximately 20 dB SPL threshold shift. At low stimulation levels LFP amplitudes were reduced. This could most likely be attributed to SS suppressive effects in the peripheral system. At high stimulation levels LFP amplitudes were enhanced. This can most likely be attributed to salicylate's inhibitory effects on GABAergic activity. (C) LFP in the lateral amygdala pre- and 2 hour post- systemic administration of SS (300 mg/kg i.p.). Results were similar to that seen in the MGB, approximately 20 dB SPL threshold shift, reduced LFP amplitudes at low stimulation levels, and enhanced LFP amplitudes with high stimulation. (D) LFP in the auditory cortex pre- and 2 hour post- systemic administration of SS (300 mg/kg i.p.). Again, the results are similar to those seen in the MGB and LA.

some compensatory increase in gain occurs after the auditory nerve to restore the IC amplitudes to their normal levels. Furthermore, electrophysiological recordings have indicated an increase in spontaneous activity in the external nucleus of the IC (eIC) following salicylate administration in guinea pigs<sup>53</sup>. In contrast, when recording from the central nucleus of the IC (ICc) in anaesthetised mice, acute salicylate administration reduced spontaneous activity in low frequency neurons<sup>54</sup> similar to what has been reported in gerbil auditory nerve<sup>44</sup>. Thus, different subdivisions of the IC appear to respond differently to high doses of SS.

#### Medial Geniculate Body (MGB)

The MGB of the thalamus is thought to play an essential role in sensory gating of auditory stimuli, and therefore

has been considered a possible contributor to tinnitus perception<sup>55</sup>. Extracellular recordings *in vitro* have indicated that salicylate can drastically alter the spontaneous firing rate of neurons in the MGB, although the direction of change is complex. Approximately 52.4% of neurons increased their firing rate after SS treatment while firing rates decreased in approximately 47.6% of neurons<sup>56</sup>. Salicylate also induces a slight increase in c-fos expression, an activity related protein, in the MGB<sup>11</sup>. In order to further evaluate the effects of salicylate in the MGB we measured the local field potentials (LFP) pre- and post-salicylate (300 mg/kg, i.p.). Figure 6-B shows the LFP I/O function to tone-bursts pre- and 2 hours post-salicylate. Salicylate induced a threshold shift of approximately 20 dB SPL, consistent with CAP threshold shifts. The LFP amplitudes were also decreased at low stimulus levels but

rapidly increased at high intensities. Preliminary recordings from multiunit clusters in the MGB also showed an overall increase in discharge rate post-SS treatment. Since the MGB provides excitatory inputs to the primary auditory cortex (A1), changes in the MGB are likely to significantly impact activity in A1.

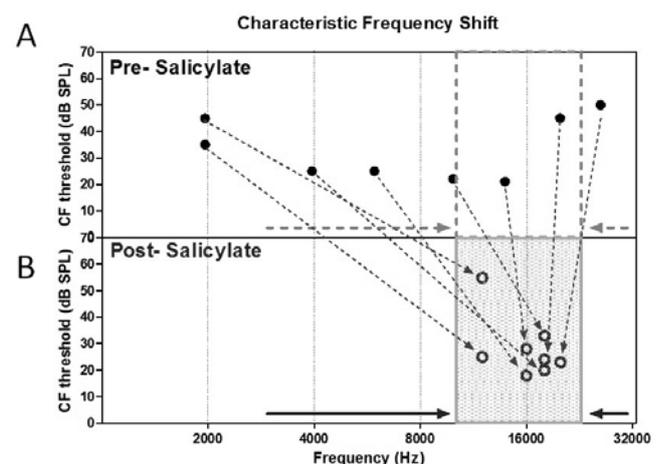
#### Auditory Cortex (AC)

The preceding results have demonstrated that not only does salicylate suppress the neural output of the peripheral auditory system<sup>5</sup>, but also alters activity in the CNS<sup>11-14</sup>. The cortex is highly plastic and shows remarkably robust changes in response to systemic salicylate as illustrated by the upsurge in c-fos immunolabelling, a marker of neural activity<sup>11</sup>. However, electrophysiological studies have found mixed results. In some cases, spontaneous firing rates in A1 and the anterior auditory field (AAF) decreased slightly post-salicylate<sup>14,30</sup> whereas the secondary auditory cortex (A2) showed an increase<sup>57</sup>. The A1 neurons mainly receive afferent inputs from the lemniscal pathway and A2 neurons receive afferent information from the extralemniscal pathway<sup>58</sup>. The reduction in A1 spontaneous firing rate following SS treatment may therefore be primarily due to the suppression of neural output from the cochlea and classical auditory pathway whereas the enhanced spontaneous firing seen in A2 may reflect the changes occurring at both auditory and non-auditory loci in the CNS. LFPs, which mainly reflect the pre-synaptic inputs, and spike discharges from multiunit clusters, which mainly reflect the outputs from AC, have been evaluated after systemic SS treatment (300 mg/kg i.p.) in anaesthetised rats. SS enhanced both the sound evoked LFP and multiunit spike discharges in the AC following systemic SS treatment<sup>13,30,59</sup>. Figure 6-D shows the sound-evoked LFP in AC as a function of stimulus intensity. At low stimulation levels the LFP is decreased and the threshold of the I/O function is elevated (shifted to the right) approximately 20 dB, which is consistent with salicylate's suppressive effects on the cochlea. In contrast to the reduced amplitudes seen in the cochlea, at high stimulation levels the amplitude of the AC LFP is enhanced compared to control amplitudes. One factor that may contribute to the enhanced AC amplitudes at suprathreshold levels is loss of GABA mediated inhibition. Evidence supporting this view comes from studies showing that systemic administration of baclofen, which increases GABA<sub>B</sub>-mediated inhibition, isoflurane anaesthesia which increases GABA<sub>A</sub>-mediated inhibition, or vigabatrin, which increases the GABA neurotransmitter concentration, can each suppress salicylate-induced hyperactivity in the AC<sup>14</sup>. These results support the hypothesis that the salicylate-induced hyperactivity seen in the AC and MGB may be due to a reduction in GABA-mediated inhibition<sup>16,18</sup>.

Under normal circumstances, GABAergic circuits help to sharpen the frequency tuning of neurons in the AC. However, when GABA-mediated activity is pharmaco-

logically suppressed frequency receptive fields (FRFs) may shift or expand. When bicuculline (BIC), a GABA<sub>A</sub> antagonist, was iontophoretically applied to the AC of chinchillas, it resulted in an expansion of neuronal frequency tuning<sup>60</sup>. The FRFs in the AC are also altered by high doses of salicylate, consistent with salicylate's effects on GABA<sup>16,18</sup>. Approximately 2.5 hours following systemic SS treatment, there was a frequency-dependent shift in CF and a widening of AC tuning curves<sup>12</sup>. This resulted in an over-representation of mid frequencies (10-20 kHz), which has previously been reported as a possible perceptive frequency for salicylate-induced tinnitus<sup>30</sup>. Figure 7-A shows the CF (x-axis) and CF threshold (y-axis) of each AC neuron pre-salicylate and Figure 7-B shows the CF and CF-threshold at 2 hours post-salicylate (300 mg/kg, i.p.). Many low-CF neurons up-shifted their CF to 10-20 kHz whereas many very high CF units down-shifted their high CF toward 10-20 kHz. The dramatic shift in FRFs in A1 could be a result of two factors. First, DPOAE and CAP data show a frequency-selective reduction in cochlear amplification that was greatest at very high and very low frequencies and least at mid-frequencies<sup>5</sup>, which may alter the FRF within A1. Second, the salicylate-induced reduction of cortical inhibition may contribute to the broadening and CF shifts of AC neurons<sup>18,60</sup>.

Similar to other regions of the neocortex, the auditory cortex is comprised of approximately six interconnected layers with a multitude of neuron types<sup>61</sup>. An *in vitro* assessment revealed significant differences in the response of different types of neurons in the AC after perfusion with 1.4 mM salicylate. The threshold current needed to evoke



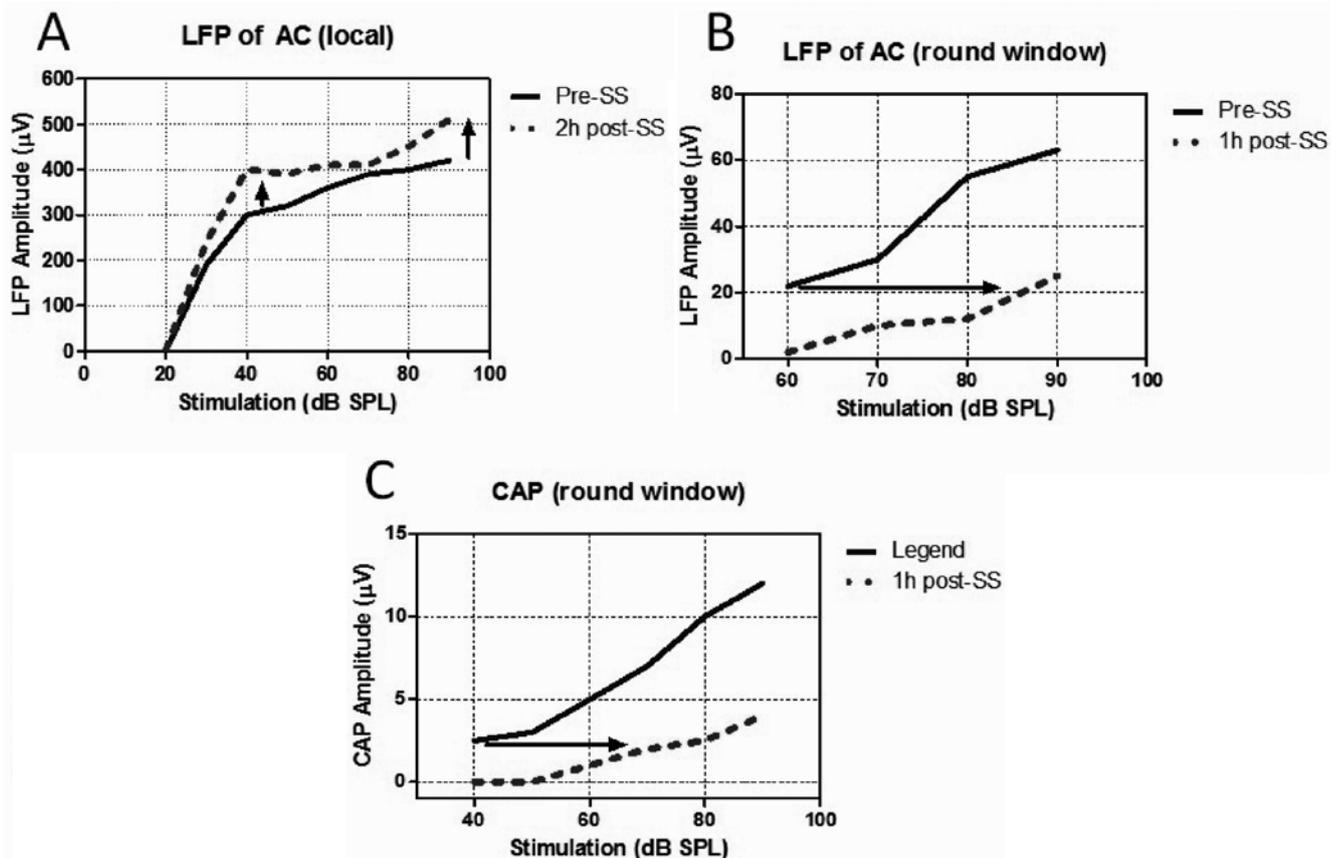
**Fig. 7.** (A) Characteristic frequency thresholds obtained from the auditory cortex prior to systemic administration of SS. (B) Characteristic frequency thresholds obtained from the same location as those displayed in panel A 2.5 hours post systemic administration of SS (300 mg/kg i.p.). After the injection, neurons shifted their frequency tuning upward and downward to the 10-20 kHz frequencies region, causing an over-representation in the mid frequencies around 16 kHz. This pitch has also been identified as a possible pitch for salicylate-induced tinnitus.

an action potential was significantly increased and current-evoked firing rates in fast-spiking interneurons were greatly depressed, however pyramidal neurons appeared unaffected<sup>62</sup>. These results indicate that salicylate preferentially impairs the function of fast-spiking GABAergic, inhibitory interneurons in specific cortical layers<sup>62</sup>. Current source density (CSD) analysis has also been used to study the effect of salicylate on stimulus-evoked LFP emanating from different layers of the AC *in vivo*. CSD analysis improves the spatial localisation of current sources (hyperpolarisation) and sinks (depolarisation) in different layers of the AC by taking into account the LFPs recorded from neighbouring electrodes. CSD analysis of sound-driven LFPs from the A1 region of the AC showed that systemic salicylate had much greater effects on some layers of the auditory cortex than others<sup>63</sup>. Under normal circumstances, CSD maps indicate a large, short latency, monosynaptic, and thalamically-driven sink in the granular layer (gSK) and a smaller, longer latency, polysynaptic, intracortically-driven sink in the supragranular layers (sSK)<sup>63</sup>. However, after systemic administration of

salicylate, the sink amplitudes in both gSK and sSK are enhanced significantly<sup>63</sup>. Additionally, the peak latency of sSK was reduced indicating more rapid processing in the supragranular layer of A1. The CSD results indicated that systemic salicylate significantly altered the intracortical microcircuits in the primary AC<sup>63</sup>.

### Local Applications of SS

To identify the central effects of salicylate independent of peripheral changes, SS was locally applied to the AC or the cochlea. Figure 8 shows the sound-driven LFP I/O functions in the AC before and after local application of SS to the cochlear round window or to the AC. When salicylate was locally applied to the round window both the CAP and AC sound-driven responses were significantly reduced and the threshold was increased approximately 25 dB<sup>13</sup> (Figure 8-B, C). However, when salicylate was applied locally to the auditory cortex (Figure 8-A) there was a significant enhancement of the sound-driven response in the AC, but no change in threshold<sup>13</sup>. Figure



**Fig. 8.** Comparison of the effect of salicylate on auditory cortex LFP pre- and post-salicylate treatment with different administration routes. (A) The effect of salicylate when locally administered to the AC surface. Local administration to the AC hyper-activates the cortex with no shift in threshold or suppressed LFPs at low stimulation levels. (B) The effect of salicylate on the AC LFP when locally administered to the round window of the cochlea. Local application to the round window results in decreased LFP amplitudes and significant threshold shift in the AC. (C) The effect on CAP when salicylate is locally applied to the round window of the cochlea. The results are similar to those seen on the LFP in AC when salicylate is applied locally to the round window. This suggests that the threshold shift and suppressed LFP amplitudes seen at low stimulation levels after systemic administration of salicylate are caused by the effects of salicylate on the cochlea and the enhanced LFP amplitudes seen at high stimulation level are a result of salicylate's influence on GABAergic neurotransmission in the CNS (i.e. LFP of AC when SS is applied locally).

6-D shows the sound-driven response from the AC when salicylate was systemically administered; the threshold shift in AC observed with systemic treatment largely originates in the cochlea whereas the hyperactivity in the AC originates in the CNS.

## Non-Classical Auditory Structures

### *Lateral Amygdala (LA)*

Interestingly, nuclei outside of the classical auditory pathway respond to acoustic stimuli, and therefore may contribute to auditory functions involved with hearing sensitivity and tinnitus perception. The amygdala, part of the limbic system, plays a role in emotional regulation and attribution of emotional significance to sensory stimuli<sup>64,65</sup>. Since tinnitus severity is often correlated with an individual's tolerance, annoyance, stress or depression<sup>66</sup>, the amygdala may play a role in tinnitus. Many neurons in the LA produce robust responses to acoustic stimuli and have good neuron frequency tuning; however, its tonotopic organisation is more complex than that of the AC<sup>19,67,68</sup>. Similar to what occurs in the AC, systemic administration of SS enhances suprathreshold, sound-driven LFPs and alters the tuning and tonotopy of FRFs<sup>19</sup>. Figure 6-C shows the I/O response of the LA before and after systemic salicylate treatment. At high intensity levels, the sound-driven response of the LA is hyperactive; however, at low intensities, the response is suppressed and threshold is elevated<sup>19</sup>. The threshold shift and suppression of low intensity sounds is a reflection of salicylate impairment of OHC amplification<sup>5</sup>. Interestingly, local application of salicylate to the LA enhanced suprathreshold, sound-driven activity in the AC, but did not alter threshold or responses to low intensity sounds in the AC<sup>19</sup>. These findings are consistent with morphological assessments showing that A1 has numerous sub-cortical pathways to areas in non-classical auditory regions such as the LA and striatum (CPu)<sup>69</sup>. Injection of bidirectional fluorescent axonal tracers into A1 of the gerbil indicated that 76% of neural pathways extend to subcortical structures while only 24% extend to cortical structures<sup>69</sup>. Taken together, these findings indicate that salicylate not only affects the cochlea, but also exerts pronounced, widespread and bidirectional effects between the central auditory pathway and other regions of the CNS. Thus, the induction of salicylate-induced tinnitus may involve aberrant neural activity within as well as outside the classical auditory pathway.

## Human perceptual deficits resulting from salicylate

Hearing sensitivity, tinnitus and supra-threshold measures of hearing are the three main perceptual alterations noticed when humans ingest large amounts of aspirin. Information obtained on the effect of large doses of aspirin

in human subjects has mainly been obtained from suicide attempts, rheumatoid arthritis patients and some psychoacoustic studies<sup>8</sup>.

## Hearing Sensitivity

Some human studies have indicated a moderate dose of aspirin can induce a hearing loss of up to ~40 dB in subjects that received 4 gm of aspirin/ day for 3-4 days<sup>22</sup>. However, other studies providing similar dosage and time periods (3.9 gm for 3-4 days) have found that the subjects only incurred an average hearing loss of ~15 dB<sup>4,8,70,71</sup>. Aspirin appears to influence hearing sensitivity across the human auditory frequency spectrum; however, most studies have neglected to evaluate hearing above 8 kHz<sup>8,72,73</sup>; and some have indicated a greater threshold shift in the high frequencies<sup>74,75</sup>. Spontaneous otoacoustic emissions in subjects that received three 325 mg tablets every six hours for 3.75 days were completely abolished<sup>76</sup>. Plasma salicylate levels seem to have a good correlation with the degree of hearing loss for serum salicylate concentrations in the range of ~60-300 mg/l<sup>22,70</sup>.

The effects of extreme doses of aspirin have been evaluated in some cases of attempted suicide. In one case, 10 gm of ingested aspirin resulted in severe hearing loss and a strong tinnitus perception within 22 hours<sup>74</sup>. DPOAEs were found to be present during aspirin intoxication; however, the responses were linearised, indicating reduced OHC function. After recovery, DPOAEs were within normal limits and showed a non-linear response pattern indicating that OHC function had been restored<sup>74</sup>. The perceptual and electrophysiological effects of extreme doses of aspirin (100 aspirin tablets) observed in a young adult male included bilateral tinnitus and hearing difficulty. Serum salicylate levels were 606 mg/l and pure tone audiometry showed a 30 dB HL bilateral hearing loss that was slightly worse in the high frequencies<sup>77</sup>. EcochG recordings made from electrodes on the promontory of the middle ear and reference electrodes on the forehead and mastoid process showed a recruiting, biphasic waveform, indicative of cochlear damage and a 50 dB threshold. One day post-ingestion, the patient reported a subjective decrease in tinnitus and an improvement in hearing sensitivity. The pure tone audiogram reverted back to normal and EcochG recordings showed a normal waveform with a threshold of 20 dB<sup>77</sup>. The previous cases suggest that aspirin can reliably impair hearing thresholds at extreme doses, but at moderate doses the effect on hearing sensitivity is more variable.

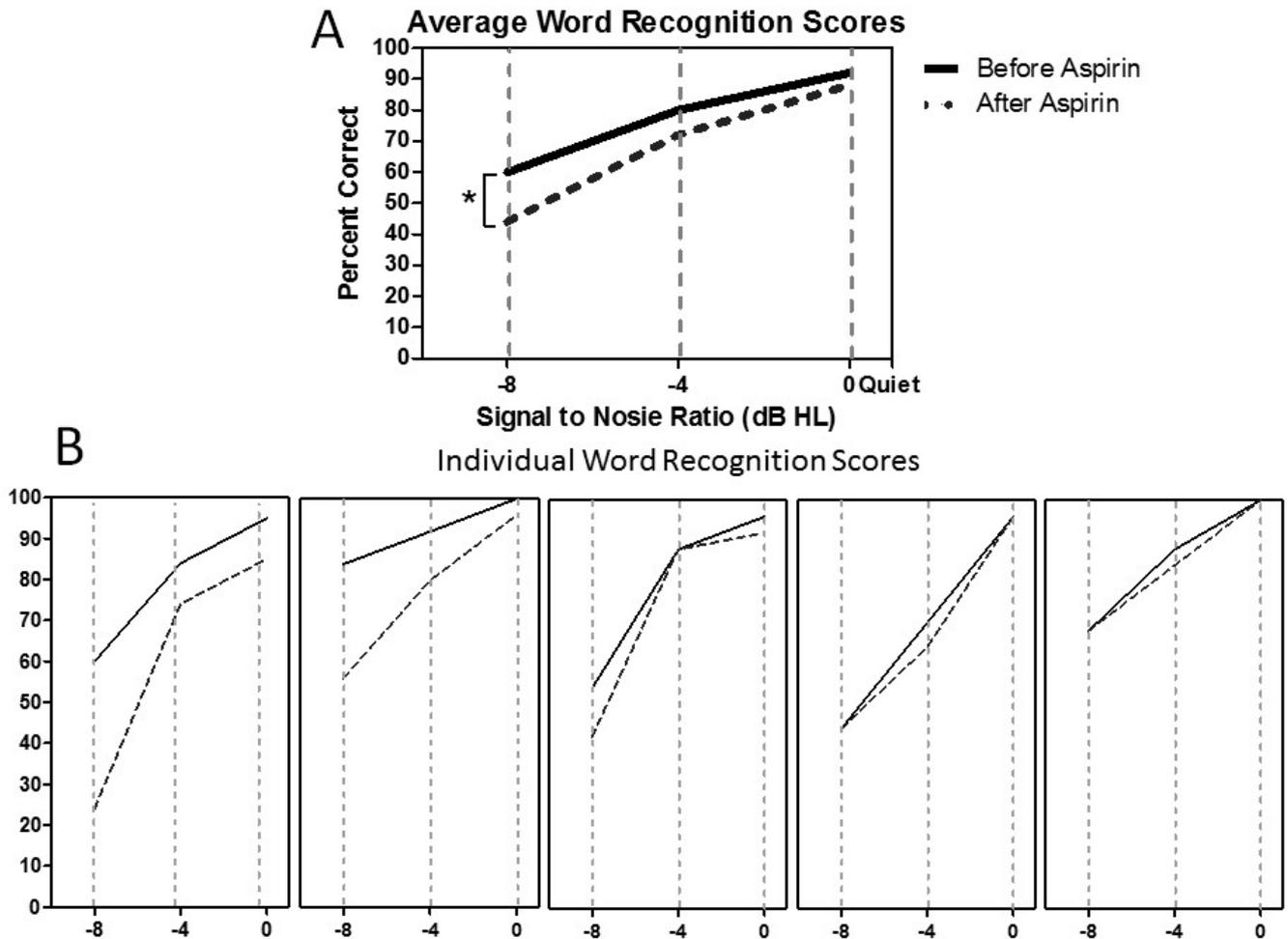
## Supra-threshold effects

It is apparent that aspirin and/or salicylate cause a sensory hearing loss. It is well known that sensory hearing loss can reduce one's ability to accurately perceive speech in noise even when the signal is presented at an individual's

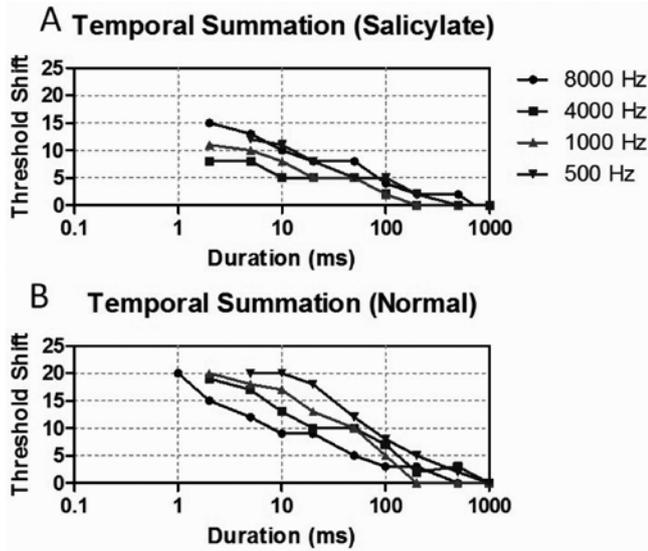
most comfortable level (MCL). Young & Wilson et al. (1982) investigated the effects of acetylsalicylic acid on speech discrimination ability in quiet and in the presence of filtered speech spectrum background noise. Measurements were obtained at three signal-to-noise ratios (SNRs 0, -4, and -8 dB HL) before and after high doses of aspirin. The average results from five subjects demonstrated a significant reduction in speech reception ability at the -8 SNR condition (Fig. 9-A). However, when the subject's scores were examined individually it was clearly apparent there was large individual variability (Fig. 9-B)<sup>20</sup>. As shown in Figure 9-B, subjects 1 and 2 both showed more difficulty discriminating speech in noise following high doses of aspirin even though they showed no significant reduction in pure tone threshold or speech discrimination in quiet following aspirin ingestion<sup>20</sup>. This study illustrates the variability that salicylate can have on auditory perception of supra-threshold stimuli.

Aside from speech discrimination aspirin can also affect temporal integration. Monaural thresholds were measured

at 500, 1000, 4000, and 8000 Hz using tone durations between 1 and 1000 msec. Fourteen subjects given 4 g/day of acetylsalicylate for 3-4 days were evaluated before, during and after salicylate treatment<sup>22</sup>. Figure 10 shows the threshold difference between long and short duration tones plotted as a function of time. Under normal conditions, the threshold of a 500 msec tone is generally 15-20 dB lower than 10 msec tones (Fig. 10-B). The improvement of threshold with increasing duration is referred to as temporal summation or temporal integration. The neural mechanism for integrating acoustic energy over time is thought to arise in a neural integration process located in the central auditory system<sup>78</sup>. Treatment with a high dose of salicylate induces a threshold elevation, but the threshold shift is greater for long duration tones than short duration tones. Consequently, the difference in threshold between a 10 msec tone and 500 msec is generally 10 dB or less (Fig. 10-A)<sup>22</sup>. The slopes of the threshold-duration functions after salicylate treatment are shallower than normal. The threshold elevation



**Fig. 9.** (A) Mean percent correct WRS in noise as a function of SNR. After a high dose of aspirin there was a significant reduction in word discrimination ability at a SNR of -8 dB HL. (B) Individual WRS in noise as a function of SNR. When averaged together aspirin appears to have a significant reduction in word discrimination ability in noise; however, when observed individually it appears that aspirin's effect on word discrimination has large variability.



**Fig. 10.** Schematic of temporal summation alterations following chronic dosing (4 g/day for 3-4 days) of acetylsalicylate. Temporal summation was tested at 500, 1000, 4000 and 8000 Hz. (A) Temporal summation ability during salicylate treatment (B) temporal summation abilities after cessation of salicylate treatment. After cessation of treatment with acetylsalicylate the slope becomes steeper indicating a better threshold for long duration tones. The effects of salicylate on temporal summation is most likely directly related to its alteration in peripheral hearing sensitivity as these results are similar to those seen in presbycusis and acoustic trauma individuals.

is thought to be due to cochlear pathology while the decrease in temporal summation is thought to be due to a change in integration processes located in the central auditory system.

Salicylate has an effect on temporal resolution or the ability to detect rapid changes in an acoustic signal. One simple measure of auditory temporal resolution is the ability to detect a short duration silent interval, or gap, in an ongoing noise. In normal hearing individuals gap detection thresholds become shorter (better temporal acuity) with increase in sound intensity reaching a minimum gap value around 60 dB SPL. To determine if salicylate would impair temporal resolution, five patients were given 3.9 gm of aspirin (salicylate) a day for a period of five consecutive days. The individuals were tested on their ability to accurately identify silent gaps in a narrow band background noise centred near 0.5 kHz or 3.5 kHz. Measurements were obtained before and during aspirin administration. After salicylate, gap thresholds became longer in four of five patients, i.e. aspirin-induced hearing loss resulted in poorer (longer gap thresholds) temporal resolution<sup>79</sup>. These results are consistent with other studies showing presbycusis and noise-induced hearing loss result in poor temporal resolution.

## Discussion

High doses of aspirin and SS have provided researchers with a powerful tool for inducing hearing loss and tinni-

tus. With short-term administration, the effects appear to be completely reversible whereas long-term administration appears to induce a unique form of damage to SGN. While salicylate and aspirin were originally believed to only affect the cochlea, more recent studies suggest that it can also have profound effects on the CNS, which should come as no surprise given that aspirin is used for relief of pain, headaches and fever. Studies of salicylate and aspirin induced ototoxicity have substantially enhanced our knowledge of auditory perception and function over the past decades and continues to be a valuable tool for investigating hearing loss and tinnitus. However, the mechanisms by which salicylate induces tinnitus, cochlear hearing loss and change the gain of the central auditory pathway are still not fully understood.

### Peripheral frequency dependency

Peripherally, salicylate suppresses the electromotile response of the OHC<sup>5</sup> by binding to anion binding sites on the motor protein prestin<sup>23</sup>. This impairs hearing sensitivity in animals and humans. Salicylate's effects on OHC amplification has frequency-dependent characteristics on DPOAE and CAP measurements in rats with the greatest suppressive effects in the low and high frequencies and the least at the mid-frequencies<sup>5 12</sup>. However, measurements in humans have shown the greatest threshold shift at high frequencies<sup>74 75</sup>. During supra-threshold testing in humans, such as speech recognition in noise, the effects of salicylate vary across individuals; some subjects show compromised speech recognition at all SNR while others show almost no effect<sup>20</sup>. The spectrum of speech is such that consonants contain primarily high frequency sounds, while vowels contain primarily low frequencies. If high frequency hearing is compromised due to salicylate ototoxicity, then it would also compromise an individual's ability to effectively discriminate the consonant speech sounds. Surprisingly, there was no apparent correlation between the severity of salicylate-induced hearing loss at high frequencies and speech recognition scores. In addition, in humans there does not appear to be a frequency-dependent effect on temporal integration abilities measured with brief tone audiometry. However, aspirin tended to have a greater effect on low frequency gap-detection threshold than high frequency gap threshold.

### Central hyperactivity and re-tuning

Salicylate's effects on the CNS seem paradoxical in light of the changes seen in the cochlea. While salicylate suppressed the neural output of the cochlea at all intensities, it enhanced LFPs and sound-driven firing rates at high intensities in the central nervous system. CSD analysis indicated that the amplified neural signal in the auditory cortex stems from changes in the intra-cortical circuits within A1<sup>63</sup>. The amplitude enhancements seen at high intensities have been well established in the AC<sup>12 13 63</sup>

and recently in non-classical auditory structures such as the amygdala<sup>19</sup>. While systemic salicylate did not lead to an amplitude enhancement in the IC, the IC responses were depressed much less than those in the cochlea. One interpretation of these results is that some signal amplification occurring between the cochlea and the midbrain partially compensates for the diminished cochlear output. Since GABAergic inhibition is present in the IC and even lower levels of the auditory pathway, any salicylate-induced reduction in GABA-mediated inhibition in the brainstem would tend to enhance the incoming signal from the brainstem<sup>13 30 80</sup>. Systemic SS treatment also induced significant CF shifts in the AC as well as the LA, which results in an overrepresentation of mid frequencies<sup>12 19</sup>. High and low frequency neurons shift their best frequencies downward and upward respectively resulting in an over representation of the mid-frequencies. The mechanisms that are responsible for the salicylate-induced CF shift are not fully understood; however, it is most likely due to two factors. One is salicylate's frequency-dependent influence in the periphery which affects mid frequencies less than high and low frequencies<sup>5</sup>. This means that the neural signal being transmitted to central auditory structures has the lowest thresholds and largest responses in the mid frequencies. Another factor is salicylate's influence on GABAergic activity. GABA plays a major role in maintaining sharp frequency tuning<sup>60</sup> and salicylate has been shown to suppress serotonin-mediated GABA inhibition<sup>18</sup>. These results suggest that the salicylate-induced CF shifts seen in the AC and LA may be the result of frequency-dependent peripheral effects and loss of centrally mediated inhibition that creates a permissive environment for re-tuning the neural circuits in the cortex.

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