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Effects of sodium salicylate on spontaneous and evoked spike rate in the dorsal cochlear nucleus

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Abstract

Spontaneous hyperactivity in the dorsal cochlear nucleus (DCN), particularly in fusiform cells, has been proposed as a neural generator of tinnitus. To determine if sodium salicylate, a reliable tinnitus inducer, could evoke hyperactivity in the DCN, we measured the spontaneous and depolarization-evoked spike rate in fusiform and cartwheel cells during salicylate superfusion. Five minute treatment with 1.4 mM salicylate suppressed spontaneous and evoked firing in fusiform cells; this decrease partially recovered after salicylate washout. Less suppression and greater recovery occurred with 3 minute treatment using 1.4 mM salicylate. In contrast, salicylate had no effect on the spontaneous or evoked firing of cartwheel cells indicating that salicylate's suppressive effects are specific to fusiform cells. To determine if salicylate's suppressive effects were a consequence of increased synaptic inhibition, spontaneous inhibitory post-synaptic currents (IPSC) were measured during salicylate treatment. Salicylate unexpectedly reduced IPSC thereby ruling out increased inhibition as a mechanism to explain the depressed firing rates in fusiform cells. The salicylate-induced suppression of fusiform spike rate apparently arises from unidentified changes in the cell's intrinsic excitability.

Keywords

Salicylate; Tinnitus; Dorsal cochlear nucleus (DCN); Patch Clamp; fusiform cell; cartwheel cell

Introduction

Hearing loss is often accompanied by tinnitus, a phantom auditory sensation whose severity varies from mild to severe. Among adults, the prevalence of tinnitus ranges from 8-15% (Coles, 1984; Henry et al., 2005; Hoffman and Reed, 2004; Nondahl et al., 2002; Snow, 1995) and for approximately 1% the symptoms are severe enough to require medical treatment (Davis and Refaie, 2000; Leske, 1981; Surveys, 1983). While many attempts have been made to identify pharmacological treatments for tinnitus, most drugs have proved ineffective (Dobie, 2004;

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Salvi et al., 2009). The development of effective drugs therapies has been hindered by a poor understanding of the biological bases of tinnitus.

Although many sites along the auditory pathway have been implicated in tinnitus (Basta and Ernst, 2004; Basta et al., 2008; Chen and Jastreboff, 1995; Eggermont and Kenmochi, 1998; Kenmochi and Eggermont, 1997; Llinas et al., 1999; Lockwood et al., 1998; Ma et al., 2006; Mahlke and Wallhausser-Franke, 2004; Sun et al., 2009; Weisz et al., 2007; Zhang et al., 2003), several lines of evidence suggest that the dorsal cochlear nucleus (DCN) plays a key role in its generation (Brozoski et al., 2002; Kaltenbach and Godfrey, 2008; Shore et al., 2007). Acoustic overstimulation, one of the most frequent causes of tinnitus, elevates spontaneous rates in tonotopic regions of the DCN associate with hearing loss (Axelsson and Ringdahl, 1989; Kaltenbach and McCaslin, 1996). The spectral profile and magnitude of the spontaneous rate increase is correlated with behavioral measures of tinnitus (Kaltenbach et al., 2004; Kaltenbach et al., 1998). Since acoustic trauma generally depress spontaneous activity in the auditory nerve, the hyperactivity observed in the DCN does not appear to originate in the cochlea (Liberman and Dodds, 1984). Moreover, DCN hyperactivity persists after cochlear ablation reinforcing the notion that the hyperactivity is not of cochlear origin (Zacharek et al., 2002). DCN hyperactivity is correlated with the amount of outer hair cell damage; however, the hyperactivity tend to be less when both inner and outer hair cells are damaged (Kaltenbach et al., 2002).

More recent experiments have linked noise-induced tinnitus with hyperactivity in DCN fusiform cells that have best frequencies tuned to the pitch of the tinnitus (Brozoski et al., 2002). Fusiform cells might represent a specific cell type involved in tinnitus initiation and a pharmacologic target for drug therapy. Fusiform cells receive auditory inputs from the cochlea via auditory nerve fibers as well as vestibular, somatosensory and higher order auditory inputs via parallel fibers originating from granule cells (Golding and Oertel, 1997; Oertel and Young, 2004). Fusiform cells receive glycinergic inhibitory inputs from cartwheel cells. Cartwheel cells receive inputs from parallel fibers and form synapses on other cartwheel cells, giant cells and fusiform cells. Fusiform cells relay their output to the inferior colliculus through dorsal acoustic stria.

High doses of sodium salicylate, the active ingredient in aspirin, induce temporary tinnitus in humans and this effect has been exploited in animal models to investigate the neural correlates of tinnitus at different sites along the auditory pathway (Basta et al., 2008; Lobarinas et al., 2004; Lobarinas et al., 2006; Myers and Bernstein, 1965; Yang et al., 2007). In a brain slice preparation of the cochlear nucleus, salicylate treatment caused spontaneous activity to increase in roughly a third of the units; decrease in another third and had no effect on the remaining third (Basta et al., 2008); however, no information was provided on the changes that occurred in specific cell types or region of the cochlear nucleus where the changes occurred. If tinnitus emerges from hyperactivity in DCN fusiform cells, then it would be important to determine exactly what effect salicylate has on fusiform cells and other major DCN cell types, especially cartwheel cells which make glycinergic inhibitory contacts on fusiform cells. Recent studies indicate that salicylate inhibits the current mediated by glycine receptors containing alpha1-subunits (Lu et al., 2009). These results suggest that salicylate might suppress glycinergic inhibitory inputs to fusiform cells thereby increasing the firing rate of fusiform cells and suppressing IPSC. To address this issue, we used the whole cell patch clamp technique to record from fusiform cells and cartwheel cells while perfusing salicylate onto brain slice preparation of the rat cochlear nucleus.

Methods

Slice preparation

SASCO Sprague Dawley rats (aged P13 – P20) were anesthetized with isoflurane and decapitated. The brainstem containing the cochlear nucleus with adjacent structures and cerebellum was cut and then glued to a cutting platform. Pseudosagittal slices (200 μm) were cut into pre-warmed (34 °C) high glucose artificial cerebral spinal fluid (HG-ACSF) containing (in mM; chemicals purchased from Sigma): 75 NaCl, 26 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 0.1 $MgCl_2$, 100 glucose, 1.36 $CaCl_2$, 4 Na L-lactate, 2 Na-pyruvate, 0.4 Na L-ascorbate, bubbled with 95% O_2 and 5% CO_2. Slices were incubated in HG-ACSF solution for at least 40 minutes, bubbled with 95% O_2 and 5% CO_2. Afterwards the temperature of the solution was gradually decreased to room temperature (~25 °C). Slices were transferred into a recording chamber with a continuous flow of fresh ACSF solution containing (in mM): 125 NaCl, 26 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 1 $MgCl_2$, 25 glucose, 1.36 $CaCl_2$ and bubbled with 95% O_2 and 5% CO_2. The recording chamber was placed under a differential interference contrast microscope (BX51WI, Olympus). Fusiform cells and cartwheel cells were identified by location within the DCN, soma shape and size, and physiological criteria. Fusiform cell were identified from their location within fusiform layer, where they are the only large cell type, apart from cartwheel cells. Fusiform cells typically have a large, spindle-shaped soma located in the deeper part of the fusiform cell layer; their apical and basal processes project towards the molecular layer and deep layer, respectively. In contrast, the cell bodies of cartwheel cells are round, generally smaller than fusiform cells, and lie close to the surface of fusiform cell layer (Golding et al., 1997). Physiologically, cartwheel cells fire unique ‘complex spikes’ that reliably distinguish them from fusiform cells, which fire simple, uniform spikes (Golding et al., 1997; Manis et al., 1994; Zhang and Oertel, 1993).

Salicylate treatment

Our standard dose of salicylate was 1.4 mM delivered for 5 minutes. This concentration was selected because it matches the level of salicylate found in cerebrospinal fluid of animals injected with 460 mg/kg (i.p.) of sodium salicylate (Jastreboff et al., 1986b); previous studies have shown that salicylate doses of 150 mg/kg (i.p.) or higher reliably produce behavioral evidence of tinnitus (Jastreboff et al., 1997; Lobarinas et al., 2006). In some experiment, 1.4 mM salicylate was applied for only 3 minutes to evaluate the extent of recovery shorter duration treatments. In a few studies, 5 mM salicylate was applied for 5 minutes to assess the effect of a higher dose.

Electrophysiology

Electrodes were pulled from glass capillary tubes (Drummond Scientific, O.D. 0.0565 inches) on a micropipette puller (Sutter, PC-84), fired polished, and wrapped with Parafilm near the tip to minimize pipette capacitance. Electrodes were filled with a potassium gluconate based internal solution containing (in mM): K-gluconate 122, NaCl 9, $MgCl_2$ 2, EGTA 0.5, HEPES 9, Tris-creatine PO_4 14, MgATP 4, Na-GTP 0.3. Whole-cell current-clamp recordings were made with an Axopatch 200B amplifier (Axon Instruments). Signals were digitized by a 16-bit data acquisition system (DIGIDATA 1320A, AXON), low-pass filtered at 5 kHz and sampled at 10 kHz. Spontaneous spikes were recorded in current clamp without applying any current. Depolarization-evoked spikes were recorded in current clamp during injection of positive current (126 pA).

Each recording session lasted 15 minutes and consisted of 15 one-minute epochs (Figure 1). Negative current (−126 pA) was injected through the electrode to keep the cells hyperpolarized except during the acquisition of spontaneous activity (6 s, 0 pA) and depolarization-evoked activity (6 s, +126 pA). Input resistances were monitored through the experiment from the

response to a hyperpolarization step between spontaneous and evoked recordings (Figure 1). Only cells with stable input resistance were included in the analysis. Spontaneous and depolarization-evoked spikes rates were calculated from the last 5 s of each of the data acquisition periods. This protocol was employed so that the cartwheel cells produced spikes with relatively uniform amplitude and interspike intervals. Baseline activity was recorded for 4 minutes (Figure 1). Then salicylate in ACSF was perfused on the slice for 3 or 5 minutes. The slice was then washed with normal ACSF until the end of the experiment.

To record IPSC, a Cs based internal solution was used which contained (in mM): CsF 50, CsCl 90, EGTA 5, QX-314 0.5, and HEPES 10. The pH was adjusted to 7.3 with CsOH. The membrane potential was held at −70 mV. The series resistance was compensated electrically with the amplifier. All currents recorded under these conditions were predominantly if not exclusively IPSC because fusiform cells do not receive noticeable spontaneous EPSC in the absence of stimulation (Zhang and Oertel, 1994). The integral of IPSC was recorded and calculated using custom software written in MATLAB 7.2. The 1.4 mM dose of salicylate was applied to each cell for 2.5 minutes followed by washout with ACSF.

Data analysis

Whole cell recordings of spike rate were evaluated from 31 fusiform cells and 7 cartwheel cells whose physiological properties remained stable over the entire recording interval. In addition, recordings of IPSC were obtained from 8 fusiform cells. Data was collected using Clampex 9.2 software and spikes were counted using custom software written in MATLAB 7.2. To allow for comparison across cells with different spike rates, each cell's spike rate was normalized to the average of the pre-salicylate baseline spike rate. Some of the fusiform cells had very low spontaneous spike rates (< 2.5 spikes/sec) or no spontaneous activity. Therefore these cells were not used in the spontaneous spike rate analysis. However, all fusiform cells were used for the analysis of evoked spike rate. Averaged values were shown ± 1 standard error.

To determine if the effects of salicylate on the spontaneous and depolarization-evoked spike rates were statistically significant, normalized spike rates recorded before the onset of salicylate application (i.e. minutes 1 to 4) were used as the control values and the values from each minute after that were used as an individual treatment (condition). Statistical analysis software (SigmaStat V3.5) was used to test for statistical significance. A one-way repeated measures ANOVA was performed to determine if there was overall group differences ($p<0.05$). For the post-hoc analysis, each time point during and salicylate treatment was compared to the control group (Mann-Whitney Rank Sum Test) to determine which values were significantly different from the control.

The experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) and were approved by University at Buffalo Institutional Animal Care and Use Committee.

Results

Fusiform cells

Figure 2 shows the spontaneous rates measured from a typical fusiform cell during the baseline period, salicylate treatment and washout with ACSF. Inserts A, B and C show typical spike waveforms recorded from the cell during baseline, salicylate and washout periods respectively. Approximately two minutes after the start of salicylate application, the spontaneous rate of the fusiform cell declined from roughly 11 Hz to 1-2 Hz. Spontaneous rate partially recovered to 5-6 Hz during the first 2-3 minutes of the washout period and remained stable over the next few minutes. The typical recording period, 15 minutes, was composed of a 4 minute baseline,

a 5 minute salicylate application period, and a 6 minute recovery. In some cases, recordings continued for longer periods post-salicylate, but there was very little recovery beyond the 6 minute recovery period.

The mean spontaneous spike rate of 6 fusiform cells was 9.95 ± 0.9 spikes/s. Figure 3A shows the mean normalized spontaneous spike rates recorded from fusiform cells (n=6) treated with 1.4 mM salicylate for 5 minutes. Salicylate caused a non-significant increase in spike rates up to minute 7 (116 ± 8%) and then the rate declined over the remainder of salicylate treatment and for the first 3 minutes of the washout period reaching a minimum of 51 ± 19%. Afterwards, spike rate gradually recovered to 86 ± 13% of baseline at 6 minutes post-treatment. Spontaneous spike rates were significantly different over the pre- and post-salicylate recording interval (one-way repeated measure ANOVA, $P < 0.001$). Spontaneous spike rates following salicylate treatment were significantly less than baseline control measures at minute 11 and minute 12 (post-hoc analysis, Mann-Whitney Rank Sum Test, $P < 0.05$).

To evaluate the effects of salicylate on evoked spike rate, 126 pA of depolarizing current was delivered through the recording electrode. Spike rate was evaluated before, during and after the 5 minute salicylate treatment in 12 fusiform cells. The mean current-evoked spike rate during baseline was 23.2 ± 1.5 spikes/s; a value approximately two times higher than the mean spontaneous rate. The mean normalized current-evoked spike rate began to decline at minute six, one minute after the start of salicylate treatment and reached a minimum of 69 ± 10% of baseline one-minute into the washout period (Figure 3B). The mean evoked rate began to recover 2-3 minutes after the start of washout and recovered to 90 ± 7% of control values 6 minutes after the start of washout. Depolarization evoked spike rates were significantly different over the pre- and post-salicylate recording interval (one-way repeated measure ANOVA, $P < 0.001$). Evoked spike rates following salicylate treatment were significantly less than baseline from minutes 8-12 (post-hoc analysis, Mann-Whitney Rank Sum Test, $P < 0.05$).

Cartwheel cells

To evaluate the generality of these findings, recordings were also obtained from cartwheel cells. In addition to their shape and location within the DCN, cartwheel cells were easily identified by their complex pike shape (inset Figure 4A). Figure 4A shows the mean (n = 7) normalized spontaneous spike rates recorded from cartwheel cells before and after salicylate treatment. Unlike fusiform cells, the mean spontaneous spike rate of cartwheel cells did not change significantly over time (one-way repeated measure ANOVA, $P > 0.05$).

The electrically evoked spike rate was also evaluated in 6 cartwheel cells before and after salicylate treatment. The mean spike rate showed a slight decrease after salicylate treatment (Figure 4B). A one-way repeated measure ANOVA indicated that the evoked rates were significantly different ($P < 0.05$) over time; however, none of the individual time points were significantly different from the baseline control rate (post-hoc analysis, Mann-Whitney Rank Sum Test, $P > 0.05$).

Effect of short-duration salicylate treatment on fusiform cells

Since the spontaneous spike rate of fusiform cells only partially recovered after 5 minute treatment with 1.4 mM salicylate, another group of fusiform cells was evaluated with a 3 minute treatment to determine if they would fully recover (Figure 5A). The spontaneous rate began to decline at the end of the 3 minute treatment period and reached at minimum of 78±17% 3 minutes into the washout period. By the end of the washout period, the spontaneous spike rate had fully recovered (102±13%). Although there was a significant difference in spontaneous rate during the recording interval (one way repeated measures ANOVA, $P < 0.05$), none of the

individual time points was significantly different from the control rate (post-hoc analysis, Mann-Whitney Rank Sum Test, $P > 0.05$).

The current-evoked spike rate was evaluated in 8 fusiform cells treated for 3 minutes with salicylate (Figure 5B). The mean current-evoked spike rate declined during the salicylate treatment, reached a minimum of approximately 81±8% of baseline 3 minutes into the washout period and fully recovered (100±5%) by the end of the washout period. There was an overall significant difference in spike rate over the recording interval (one way repeated measure ANOVA, $P< 0.05$). A post-hoc analysis showed that the evoked spike rates at minute 9 and minute 10 were significantly different from control (post-hoc analysis, Mann-Whitney Rank Sum Test, $P < 0.05$). These results indicate that both the spontaneous and evoked spike rates of fusiform cells fully recover following a short, 3 minute treatment with 1.4 mM salicylate.

Effect of high dose salicylate on fusiform cells

Spontaneous spike rates were recorded from 5 fusiform cells (only 5 of 10 fusiform cells were spontaneously active) during 5 minute treatment with 5 mM salicylate. The mean normalized spontaneous rate showed a noticeable increase after the start of salicylate application (Figure 6A). The mean spike rate reached a peak of 148 ± 49% at minute six and then declined moderately during the remainder of the treatment. During the washout period, the mean spike rate decreased to a minimum of 65 ±18 % of baseline at minute 13. Afterwards, the spontaneous rates started to recover and reached 81 ± 18% of baseline at the end of the wash out period. However, statistical analysis showed that these changes were not significant (one way repeated measures ANOVA; $P > 0.05$) presumably due to the large variance.

Spike rates evoked by current injection were recorded from 10 fusiform cells during 5 minute treatment with 5 mM salicylate (figure 6B). During the first 2 minutes of treatment, the average evoked spike rate increased slightly to 112 ± 7%. Afterwards, the evoked spike rate fell below the baseline reaching a minimum value of 61 ± 12% at minute 10 (1 minute into the washout period). Afterwards, the evoked spike rate gradually recovered to 97±4% of baseline by the end of the washout period. There was a significant difference in evoked rate over the recording period (one way repeated measures ANOVA; $P < 0.05$). The evoked spike rates at minutes 9 and 10 were significantly different from baseline control values (post-hoc analysis, Mann-Whitney Rank Sum Test, $P < 0.05$).

Fusiform cell IPSC

To determine if the decrease in spontaneous and evoked spike rate seen in fusiform cells was the result of increased inhibition, we measured the spontaneous IPSC before and after salicylate treatment. Figure 7 shows the normalized integral of the IPSC from 8 fusiform cells over the recording period. The integral of the IPSC showed little change during salicylate treatment and a moderate decrease (maximum decrease ~15%) during salicylate washout. There was a significant difference in the integral of the IPSC over the recording period (one way repeated measures ANOVA; $P < 0.05$). The integral of the IPSC during the entire post-treatment period (minutes 4.5-8) was significantly less than baseline control values (post-hoc analysis, Mann-Whitney Rank Sum Test, $P < 0.05$).

Discussion

High doses of salicylate have previously been reported to alter the spontaneous activity of cochlear nucleus neurons in vitro; spontaneous activity increased in roughly a third of the units, decreased in another third and was unaffected in the remainder. However, it was unclear what regions of the cochlear nucleus were assessed and what cell types were affected by salicylate treatment (Basta et al., 2008). The present study, carried out with physiologically relevant

salicylate concentrations (Boettcher et al., 1990; Jastreboff et al., 1986b; Jastreboff et al., 1997), identifies for the first time the specific effects salicylate has on the two major cell types in the DCN, namely fusiform cells and cartwheel cells. Five minute application of 1.4 mM salicylate suppressed both spontaneous and depolarization evoked spike rates in fusiform cells; the suppressive effects of salicylate partially recovered during the 6 minute washout period. The same general trends were observed with a shorter duration treatment (3 minutes) or a higher concentration (5 mM) of salicylate. In contrast, the same 1.4 mM dose of salicylate had no effect on the spontaneous and depolarization evoked spike rates of cartwheel cells. The first two findings indicate that the effects of salicylate are specific to fusiform cells and are not the result of a global (e.g., toxicity, pH, osmolarity) effect of salicylate on the DCN.

Spontaneous hyperactivity in the DCN is believed to arise from a shift in the balance of excitation and inhibition (Brozoski et al., 2002; Brozoski et al., 2007; Kaltenbach, 2007; Kaltenbach and Zhang, 2007). Fusiform cells receive strong glycinergic inhibitory inputs from cartwheel cells (Backoff et al., 1997; Juiz et al., 1996; Kaltenbach et al., 2005; Kaltenbach et al., 2002; Moore et al., 1996; Mugnaini, 1985; Rubio and Juiz, 2004), and recent evidence indicates that salicylate can inhibit glycine receptors containing alpha1-subunits (Lu et al., 2009). Therefore, we speculated that salicylate might increase the firing rate of fusiform cells by suppressing IPSC. However, 5 minute application of 1.4 mM salicylate unexpectedly reduced the spike rates of fusiform cells. Considering the facts that the spontaneous rates of cartwheel cells were unaffected by salicylate and IPSC in fusiform cells were slightly decreased, the salicylate-induced reduction of fusiform cell spontaneous activity cannot be due to an increase of inhibition.

High doses of salicylate have been reported to increase spontaneous activity in the guinea pig inferior colliculus (Jastreboff and Sasaki, 1986a). This raises the possibility that the changes seen in the inferior colliculus are initiated by hyperactivity originating in the DCN. However, our results conflict with this hypothesis since salicylate either suppressed or had no effect on the spontaneous rates of fusiform and cartwheel cells respectively. Similarly, suppression of activity in putative fusiform cells has been reported after intense noise exposure (Chang et al., 2002). The down regulation of spontaneous activity seen in fusiform cells after noise and salicylate could perturb activity either within local or remote neural networks and lead to tinnitus. Our salicylate data, however, are compatible with more recent in vitro data showing that salicylate suppresses spontaneous activity in the mouse inferior colliculus (Ma et al., 2006) and other more rostral sites in the auditory pathway (Yang et al., 2007). Collectively, these data suggest salicylate-induced tinnitus does not arise from hyperactivity in fusiform or cartwheel cells in the DCN, but from some other mechanism or from some other site in the central auditory pathway.

Significant increases in spontaneous activity typically appear in the DCN after acoustic overstimulation or cisplatin treatment (Brozoski et al., 2002; Kaltenbach et al., 2005; Melamed et al., 2000; Rachel et al., 2002). These physiological results along with relevant behavioral metrics suggest that tinnitus may be related to spontaneous hyperactivity in the DCN and that the axons of fusiform cells relay this information to the inferior colliculus via the dorsal acoustic stria (Brozoski et al., 2002; Cant and Benson, 2003; Imig and Durham, 2005; Kaltenbach et al., 2008; Melamed et al., 2000; Shore et al., 2007). However, recent study showed that selective ablation of DCN did not abolish the psychophysical evidence of tinnitus or actually increase it in some cases (Brozoski and Bauer, 2005). Our results also do not support a *local mechanism* of action for salicylate in the DCN hyperactivity model of tinnitus since we found salicylate quickly suppressed spontaneous rates in fusiform cells and had no effect on cartwheel cells. More complex models of salicylate-induced tinnitus may be required in which salicylate disrupts neuronal function at multiple sites along the auditory pathway perhaps by increasing the gain of the central auditory system (Lu et al., 2009; Sun et al., 2009; Wang et al., 2008).

There are several possible explanations why we may not have observed hyperactivity in the DCN after salicylate treatment. Our in vitro brain slice preparation eliminates many of the local and descending inputs to the DCN as well as inputs from the cochlea; the physiological effects of salicylate on DCN spontaneous activity could conceivably be fundamentally different. In addition, we only recorded from fusiform cells, whose axons project out of the cochlear nucleus, and cartwheel cells, that inhibit fusiform cells. The salicylate-induced hyperactivity observed in the cochlear nucleus by others could arise from other cell types we did not record from (Basta et al., 2008). High doses of salicylate also affect the cochlea; several studies report that salicylate increases the spontaneous rates of auditory nerve fibers (Evans and Borerwe, 1982; Ruel et al., 2008) while others have reported the exact opposite (Muller et al., 2003). Since the spiral ganglion neurons are absent from our brain slice preparation, the peripheral effects of salicylate were eliminated from our recordings. Despite these limitation, the local effects of salicylate observed in our DCN preparation provide important new information on the effect that salicylate has on fusiform and cartwheel cells, IPSC and the role the DCN plays in salicylate-induced tinnitus. The local effects of salicylate seem pertinent to models of tinnitus involving the DCN and other models that posit a central origin since DCN hyperactivity and tinnitus persist even after cochlea ablation (Coad et al., 2001; Zacharek et al., 2002).

Salicylate selectively suppressed spontaneous and evoked spike rate in fusiform cells, but not cartwheel cells. This cell-specific effect is presumably related to some unique, but unidentified properties of fusiform cells. Salicylate has been reported to affect many different types of ion channels including L-type calcium channels, potassium channels and sodium channels (Liu and Li, 2004; Liu et al., 2005a; Liu et al., 2005b). In addition, salicylate also influences numerous types of receptors such as NMDA, GABAergic, serotonergic and glycinergic receptors (Bauer et al., 1999; Finlayson and Kaltenbach, 2009; Milton et al., 2009; Peng et al., 2003; Ruel et al., 2008; Wang et al., 2006). Therefore, the salicylate-induced suppression of fusiform firing rates could be caused by the combined effect of one or more these ion channels or receptors as well as other unknown factors.

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Abbreviations

DCN	dorsal cochlear nucleus
IPSC	inhibitory postsynaptic current
HG-ACSF	high glucose artificial cerebral spinal fluid
SS	sodium salicylate

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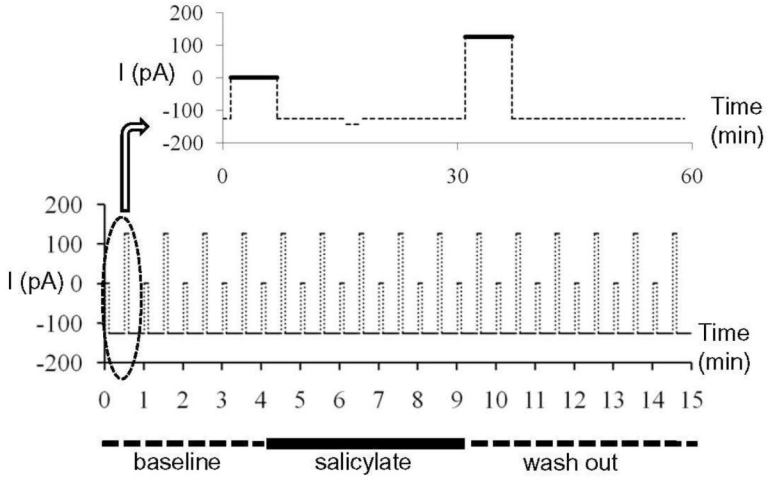

Figure 1.

Protocol for current clamp recording. During current clamp recording, cell injected with −126 pA except during acquisition of spontaneous and evoked spike rate when the current was 0 and +126 pA. A hyperpolarization current step was performed between two recording periods to monitor the input resistance. Salicylate was applied for 5 minutes starting at minute 5, followed by a wash out period.

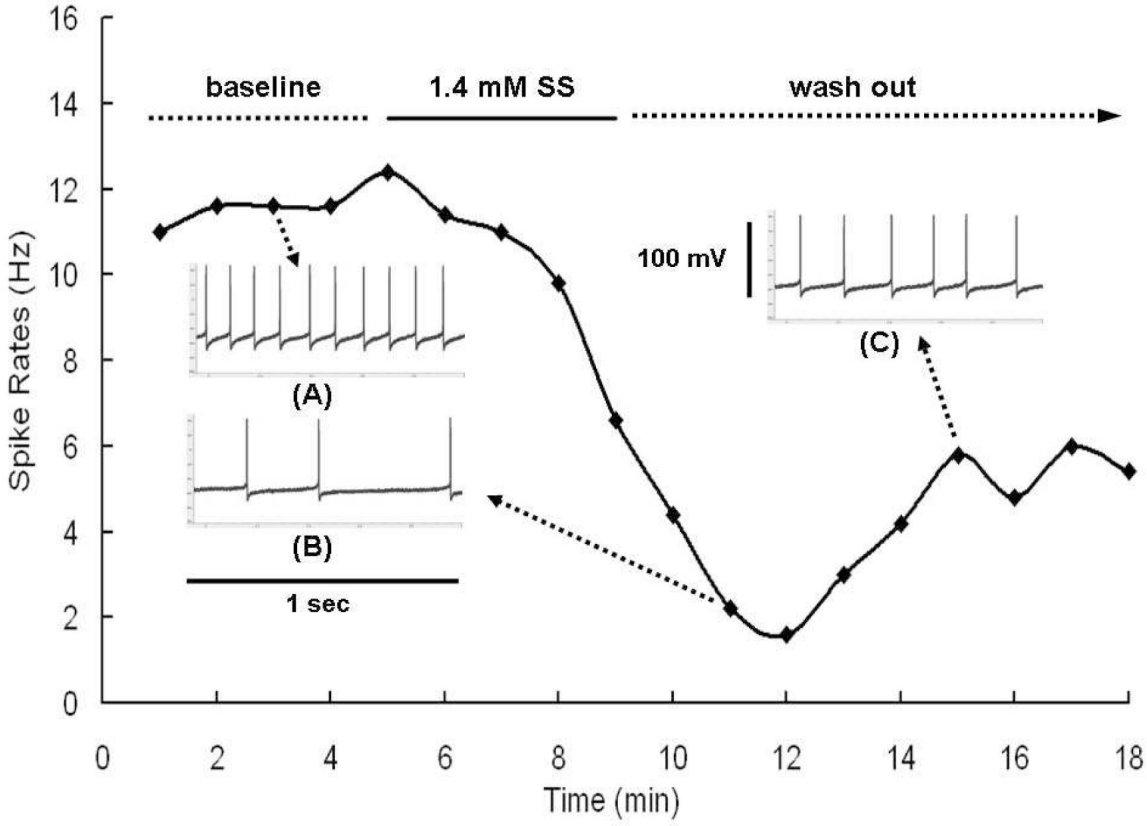

Figure 2.
A sample plot of spontaneous spike rate of fusiform cell obtained during 5-s intervals over baseline, during 5 minute treatment with 1.4 mM sodium salicylate (SS) and during the washout period. Inserts A, B, and C show typical spiking pattern observed during 1-s intervals during baseline, SS and washout periods.

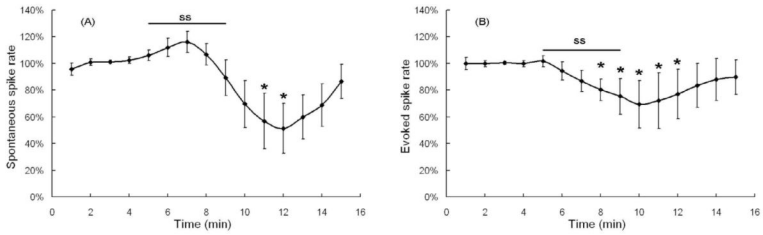

Figure 3.

Effect of 5 minute treatment with 1.4 mM salicylate (SS) on fusiform cells. (A) Mean spontaneous spike rates (N=6; +/− SE). (B) Mean rate evoked by a +126 pA depolarization current (N=12; +/− SE). The curve shows the average of normalized spike rates across all cells recorded under this condition. * indicates points significantly different from baseline, which is a set of normalized spike rates during the first 4 minutes of each cell.

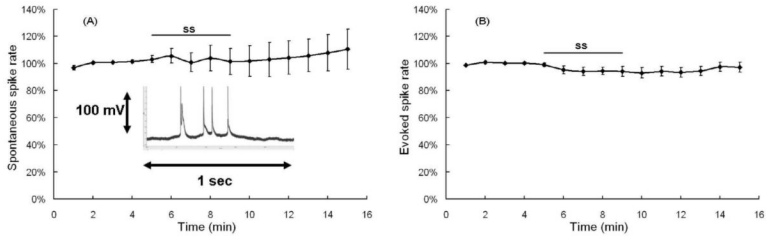

Figure 4.

Effect of 5 minutes of 1.4 mM salicylate (SS) on cartwheel cells. Typical 'complex spikes' used to physiologically classify cartwheel cells. (A) Mean spontaneous spike rates (N=7, +/− SE). None of the individual post-treatment points were significantly different from baseline. (B) Mean rate evoked by a +126 pA depolarization current (N=6, +/− SE). None of the individual post-treatment points were significantly different from baseline.

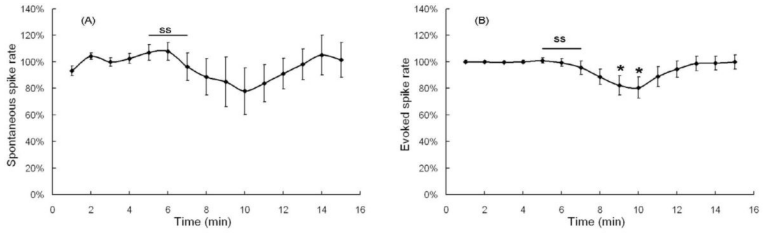

Figure 5.

Effect of 3 minutes of 1.4 mM sodium salicylate (SS) on fusiform cells. (A) Mean spontaneous spike rate (N=5; +/− SE); none of the individual points were significantly different from baseline. (B) Mean spike rates evoked by +126 pA depolarization current (N= 8; +/− SE); none of the individual points were significantly different from baseline.

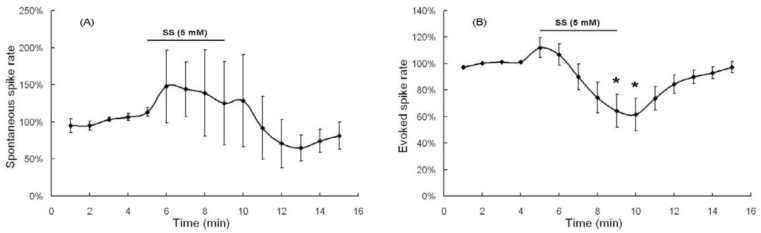

Figure 6.

Effect of 5 minutes of 5 mM sodium salicylate (SS) on fusiform cells. (A) Mean spontaneous spike rate (N= 5; +/− SE); none of the individual points were significantly different from baseline. (B) Mean fusiform spike rate evoked by +126 pA depolarization current (N= 10; +/− SE); * indicate points significantly difference from pre-SS baseline.

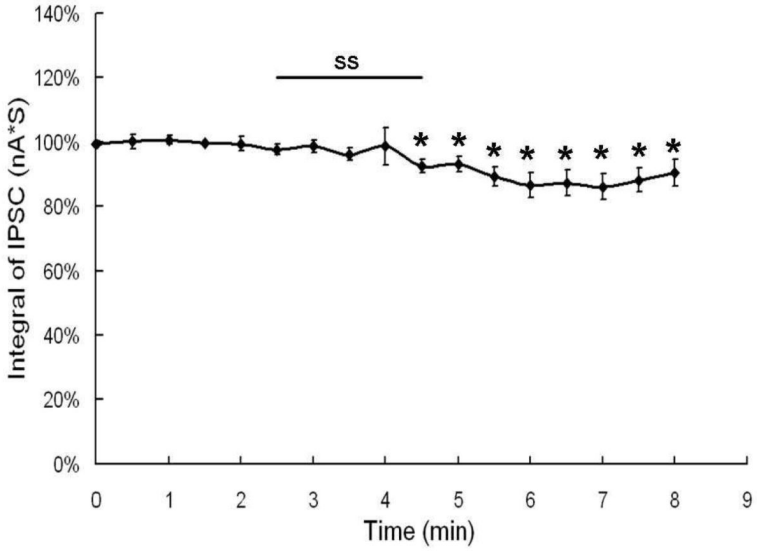

Figure 7.
Effect of 1.4 mM sodium salicylate (SS) on mean (n=8; +/− SE) of the integral of IPSC recorded from fusiform cells; * indicate points significantly difference from pre-SS baseline.