Expression of tumor necrosis factor-α and interleukin-1β genes in the cochlea and inferior colliculus in salicylate-induced tinnitus

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Abstract

Background: Changes in the gene expressions for tumor necrosis factor-α (TNF-α) and/or interleukin-1β (IL-1β) during tinnitus have not been previously reported. We evaluated tinnitus and mRNA expression levels of TNF-α, IL-1β, and N-methyl D-aspartate receptor subunit 2B (NR2B) genes in cochlea and inferior colliculus (IC) of mice after intraperitoneal injections of salicylate.

Methods: Forty-eight 3-month-old male SAMP8 mice were randomly and equally divided into two groups: salicylate-treated and saline-treated. All mice were trained to perform an active avoidance task for 5 days. Once conditioned, an active avoidance task was performed 2 hours after daily intraperitoneal injections of saline, either alone or containing 300 mg/kg sodium salicylate. Total numbers of times (tinnitus score) the mice climbed during the inter-trial silent period for 10 trials were recorded daily for 4 days (days 7 to 10), and then mice were euthanized for determination of mRNA expression levels of TNF-α, IL-1β, and NR2B genes in cochlea and IC at day 10.

Results: Tinnitus scores increased in response to daily salicylate treatments. The mRNA expression levels of TNF-α increased significantly for the salicylate-treated group compared to the control group in both cochlea (1.89 ± 0.22 vs. 0.87 ± 0.07, P < 0.0001) and IC (2.12 ± 0.23 vs. 1.73 ± 0.22, p = 0.0040). mRNA expression levels for the IL-1β gene also increased significantly in the salicylate group compared to the control group in both cochlea (3.50 ± 1.05 vs. 2.80 ± 0.28, p < 0.0001) and IC (2.94 ± 0.51 versus 1.24 ± 0.52, p = 0.0013). Linear regression analysis revealed a significant positive association between tinnitus scores and expression levels of TNF-α, IL-1β, and NR2B genes in cochlea and IC. In addition, expression levels of the TNF-α gene were positively correlated with those of the NR2B gene in both cochlea and IC; whereas, the expression levels of the IL-1β gene was positively correlated with that of the NR2B gene in IC, but not in cochlea.

Conclusion: We conclude that salicylate treatment resulting in tinnitus augments expression of the TNF-α and IL-1β genes in cochlea and IC of mice, and we suggest that these proinflammatory cytokines might lead to tinnitus directly or via modulating the NMDA receptor.

Background

Tinnitus is the perception of sound in the absence of acoustic stimulation. Usually occurring together with hearing loss, tinnitus can be perceived in one or both ears, or in the head. Tinnitus is distinguishable from auditory hallucinations, which involve hearing one or more talking voices. Salicylate-induced tinnitus in mice has been a popular animal model for the study of tinnitus [1]. The mechanism of salicylate-induced tinnitus is postulated to involve accumulation of arachidonic acid caused by inhibition of cyclooxygenase (COX) that could potentiate N-methyl D-aspartate receptor (NR) currents at synapses between inner hair cells and dendrites of the cochlear spiral ganglion neuron [2].

Tinnitus-related changes in gene expression have been recently reported. Microarray studies have revealed that 87 genes are up-regulated and 140 genes are down-regulated by two-fold or more in mouse cochlea during...
salicylate ototoxicity [3]. Jia and Qin [4] have reported that expression of c-fos and NR2A increases in auditory cortexes of rats that experience tinnitus after salicylate injection. We also have found that mRNA expression levels for COX-2 decrease slightly, whereas expression levels of NR2B increase moderately in cochlea and mid-brain of salicylate-treated, senescence accelerated prone mice strain 8 (SAMP8 mice) [5].

Recent studies have shown that inflammatory responses occur in the inner ear under various damaging conditions, including overstimulation with noise [6] and cisplatin-induced ototoxicity [7]. However, an association between proinflammatory cytokines and tinnitus has been rarely reported. In chronic tinnitus sufferers, a relaxation training program can result in significantly decreased stress, anxious depression, anger, and tinnitus disturbance, paralleled by a reduction of TNF-α, but not IL-6 or IL-10 [8]. Also, previous studies have shown that TNF-α and IL-1β might interact with the NR [9-12] in inflammatory hyper-algesia [13], bone cancer pain [14], seizures [15], vision loss [16], and spinal cord injury [17]. Because the NR is also linked to tinnitus in rats [2] and mice [5], we hypothesized that tinnitus may be associated with changes in gene expression of these proinflammatory cytokines.

Apart from the “theory of cochlear origin” [18], tinnitus is most commonly believed to originate from the central nervous system. Positron emission tomography has shown increased metabolic activity consistent with neuronal activation in the inferior colliculus (IC) and in the auditory cortices of rats with salicylate-induced tinnitus [19]. Thus, we sought to assess gene expressions in cochlea and IC for this study.

**Methods**

**Animals**

Because tinnitus is most prevalent in older humans, we used the SAMP8 mice for this study, although other strains of rodents, cats, or rabbits could also have been used. Forty-eight 3-month-old male SAMP8 mice, weighing between 22 and 33 g, were randomly and equally divided into two groups: salicylate-treated (n = 24) and saline-treated (n = 24). Our Institutional Animal Care and Use Committee approved the protocols used in this study.

**Behavioral measurement of tinnitus score**

All mice were trained to perform an active avoidance task, which was performed in a conditioning box with an electrical floor and a climbing pole, according to the design of Guitton et al. [2].

**Conditioning to the task**

The conditioning paradigm consisted of 6 sessions performed daily for 5 days (day 1 to 5). Each session lasted 15 to 20 min, and there were 10 trials per session. Inter-trial intervals were at least 1 minute. For each trial, the conditioning stimulus was a 50 dB sound pressure level (SPL) pure tone with a frequency of 10 k Hz and a 3-second duration. The unconditioned stimulus was a 3.7 mA electric foot-shock presented for up to 30 seconds, as described by Guitton's protocol [2], by adjusting the electric voltage with fixed copper wire resistance on the floor. The time between the conditioned stimulus and the unconditioned stimulus was 1 second. The mice would climb up the pole to a safe area after the coupled conditioned and unconditioned stimuli. Electrical shocks were stopped by the experimenter when the animal climbed correctly. The “true-positive” score was the level of performance as assessed by the number of times the mice climbed correctly in response to sound. Mice were considered to be conditioned when the “true-positive” score reached at least 80% in three consecutive sessions. Only conditioned mice entered the tinnitus experiments.

**Induction and testing of tinnitus**

Once conditioned, the mice rested for 1 day (day 6). Then, for 4 consecutive days (days 7 to 10), an active avoidance task was performed 2 hours after intraperitoneal injections of saline either alone or containing 300 mg/kg sodium salicylate (Sigma, St. Louis, MO) [1,2,20]. The active avoidance task consisted of one session with 10 trials. To compensate for hearing loss (threshold elevation of 15 to 20 dB SPL during 4-days injections in our preliminary, unpublished data) induced by salicylate injection, the intensity of sound that elicited the behavioral responses was increased to 70 dB SPL for the salicylate-treated group. By doing so, the sound sensation levels of all mice in both groups were similar.

During testing, a sound of 3-second duration was given and the mice were observed for another 5 seconds to see whether they would climb to the safe area as conditioned (true-positive). If the animals stayed in the safe area ≥10 seconds, the mice were returned to the floor for ongoing observation. If the mice did not climb to the safe area in response to the sound, an electrical shock was given by the experimenter to remind the mice to climb to the safe area. Again, if animals stayed in the safe area ≥10 sec, the mice were returned to the floor for ongoing observation. Finally, the experimenter observed the total number of times (tinnitus score) the mice climbed during the inter-trial silent period of 1 minute (false-positive climbs) for 10 trials.

**Samples isolation and RNA extraction from cochlea and IC**

The body weights of all mice were measured at day 7 before salicylate or saline injection, and at day
10 before the mice were euthanized by decapitation. The weights of the midbrain including IC were also measured before dissection. Paired samples of cochlea and IC were immediately dissected using a Zeiss stereomicroscope and stored in a -80°C freezer until use. RNA isolation was performed using RNA-Bee isolation reagent (Friendswood, USA) with a tissue homogenizer according to the manufacturer’s protocol. RNA quality was assessed using an Agilent Bioanalyzer 2100 and the ratio of absorbance measurements at 260 and 280 nm.

**Reverse transcription-polymerase chain reaction (RT-PCR)**

High quality RNA was used as substrate to synthesize cDNA by reverse transcription using a MasterAmp™ High Fidelity RT-PCR Kit (Epicentre Biotechnologies, USA) in a P × 2 Thermal cycler (Thermo Electron Corporation Bioscience Technologies Division, USA). RT was carried out at 37°C for 1 hour. For PCR amplification, 7.5 μL cDNA and primers were used according to the supplier’s instructions. The primers were: TNF-alpha-F, 5’-CCCCCTCAGCAACCCAC-CAAG-3’, TNF-alpha-R, 5’-CTTGGCAGATTGACCTCA GC-3’; IL-1beta-F, 5’-GAGTGTGGATCC CAAGCAAT-3’, IL-1beta-R, 5’-CTCAGTGGAGCTATGACCA-3’; NMDA receptor subtype 2B (NR2B)-F, 5’-TCC GCC GAG AGT CCT CCG T-3’, NR2B-R, 5’-CTG CGT TGC CCT CGA TGT T-3’; β-actin-F, 5’-CCACACCCGGCCACAGTTCG-3’, and β-actin-R, 5’-CCCATTCCCACCATCACACC-3’ (Protech-taiwan, Taiwan).

The thermal cycling conditions for PCR were adjusted to the following: 3 min initial set-up at 95°C; followed by 50 cycles of denaturation (45 s at 95°C for all genes), annealing (45 s at 53°C for TNF-alpha, 52°C for IL-1beta, 54°C for NR2B, and 50°C for β-actin). A final 10 min extension at 72°C was included for all thermal cycle runs.

**Quantification of PCR products**

The DNA products were measured using a Mini Horizontal Electrophoresis System (MJ-105/MP-100, Major Science, Taiwan) and an E-Box-1000/26M Inspection Certificate & Analysis Systems (E-Box Spp-010 E-capt soft ware, USA). The expression levels of TNF-α, IL-1β and NR2B genes are presented as relative ratios in comparison to β-actin.

**Statistical analysis**

Data are presented as mean ± standard deviation (SD), unless indicated otherwise. Expression levels for the TNF-α or IL-1β genes, presented as ratios relative to β-actin, were compared for both groups using Student’s t-test. The correlation between the tinnitus score (dependent variable) and TNF-α or IL-1β gene expression was analyzed using a linear regression model. All of the above analyses were performed using the commercialized software “STATA10”, and p values < 0.05 were considered statistically significant.

**Results**

The body weight of each mouse did not change significantly during active avoidance task conditioning when performed according to Guitton’s design [2]. The mean body weight before injection of intraperitoneal salicylate was 28.4 ± 1.9 g for the salicylate group and 29.0 ± 1.2 g for the control group (p = 0.1969). On day 4 of tinnitus induction, mean body weights were still not significantly different (p = 0.255) between salicylate-treated mice (29.4 ± 1.24 g) and control mice (28.8 ± 1.9 g). In addition, the weights of the midbrain including IC were not significantly different between the two groups (0.3 ± 0.03 g salicylate group, 0.3 ± 0.02 g control group, p = 0.6895).

Tinnitus scores, defined as the total number of times the mice climbed to the safe area during the inter-trial silent period of 1 minute (false positive climbs) for 10 trials, increased between day 1 and day 4 of intraperitoneal injections for salicylate-treated mice but did not increase in the control group. The mean tinnitus score at day 4 was 0.5 ± 0.5 for the control group and was 8.0 ± 1.5 for the salicylate group.

Figure 1 shows the gene expression levels for TNF-α in cochlea and IC at day 4 after tinnitus induction. In the salicylate group compared to the control group, the mean expression level for TNF-α increased significantly in both cochlea (1.89 ± 0.22 vs. 0.87 ± 0.07 respectively, p < 0.0001) and in IC (2.12 ± 0.23 vs. 1.73 ± 0.22 respectively, p = 0.0040). Figure 2 shows expression levels for
the IL-1\(\beta\) gene in cochlea and IC at day 4 after tinnitus induction. In the salicylate group compared to the control group, the mean expression level for the IL-1\(\beta\) gene increased significantly in cochlea (3.50 ± 1.05 vs. 2.80 ± 0.28, respectively, \(p < 0.0001\)) and in IC (2.94 ± 0.51 vs. 1.24 ± 0.52, \(p = 0.0013\)).

Table 1 addresses the relationships between tinnitus scores and gene expression levels for TNF-\(\alpha\), IL-1\(\beta\), and NR2B. Linear regression analysis showed that tinnitus scores were positively associated with gene expression level of NR2B and with gene expression levels of TNF-\(\alpha\) and IL-1\(\beta\) in cochlea and IC (see Table 1 for data).

In addition, linear regression analysis showed that the gene expression level of TNF-\(\alpha\) was positively associated with NR2B gene expression in cochlea (\(\beta \pm SE = 1.07 \pm 0.14\), \(p < 0.001\)) and in IC (\(\beta \pm SE = 0.93 \pm 0.28\), \(p = 0.001\)). Similarly, the gene expression level of IL-1\(\beta\) was positively associated with NR2B gene expression in IC (\(\beta \pm SE = 0.24 \pm 0.083\), \(p = 0.005\)), but not in cochlea (\(\beta \pm SE = 0.08 \pm 0.135\), \(p = 0.575\)).

Discussion

Considering the results of Weber et al. [8], we suggested that proinflammatory cytokines, in addition to their contributions to hearing impairment [6,7], might also play an important role in the pathophysiology of tinnitus. This experimental study showed that mRNA levels of the TNF-\(\alpha\) and IL-1\(\beta\) genes increased both in cochlea and IC after intraperitoneal injection of high dose salicylate. In addition, the tinnitus scores showed significant positive associations with the gene expression levels of TNF-\(\alpha\), IL-1\(\beta\), and NR2B in both cochlea and IC.

Previous studies have focused on the roles of proinflammatory cytokines in hearing impairment. For example, virally encoded macrophage inflammatory proteins appear to play a significant role in cytomegalovirus-related hearing loss [21]. Cochlear immunohistochemistry has confirmed the presence of numerous cytokines, as well as NF-kB, in otitis media-induced cochlear cytotoxicity [22]. TNF-\(\alpha\), IL-1\(\beta\)a and IL-6 are significantly induced in the lateral side of the spiral ligament and stria vascularis early after noise exposure. Furthermore, IL-6 is detected in spiral ganglion neurons at 12 and 24 hr after noise exposure. These cytokines may initiate an inflammatory response and lead to noise-induced cochlear damage [6,23]. In addition, TNF-\(\alpha\), IL-1\(\beta\) and IL-6 are associated with cisplatin-induced ototoxicity [7]. However, infusion of TNF-\(\alpha\) into the cochlea of guinea pigs does not result in obvious hearing loss, although inflammatory cells are recruited to the cochlea [24]. The results of this study, together with those of Weber et al. [8], suggest that proinflammatory cytokines might also account for the induction of tinnitus, but the exact mechanisms involved are still unclear.

Tinnitus can be regarded as phantom auditory pain, which is often followed by peripheral hearing loss [25]. In chronic neuropathic pain studies, TNF-\(\alpha\), IL-1\(\beta\) and IL-6 have been reported to induce central sensitization and hyperalgesia after nerve injury via distinct and overlapping synaptic mechanisms in superficial dorsal horn neurons. Furthermore, these proinflammatory cytokines may induce long-term synaptic plasticity through CREB-mediated gene transcription [26-30]. It is reasonable to hypothesize that these cytokines might also contribute to tinnitus via mechanisms similar to those of post-traumatic neuropathic pain.

Tinnitus has been reported to be highly associated with NMDA receptor activity [2] and/or its gene expression [5]. Several studies have reported an interaction between TNF-\(\alpha\) and/or IL-1\(\beta\) and functions of NMDA receptor [9-17]. Conversely, NMDA can also increase expression of TNF-\(\alpha\) and IL-1\(\beta\), as well as endothelial
adhesion molecules, including ICAM-1, and induce leukocyte accumulation in retinal vessels and retina ganglion cell death [16]. Glutamate could influence the release of cellular proinflammatory responses in arthritis [12]. Therefore, it is also reasonable to hypothesize that TNF-α and IL-1β might contribute to tinnitus via augmenting NMDA receptor expression and/or its functions.

**Conclusion**

These data demonstrate that salicylate-induced tinnitus correlates with increased gene expression of TNF-α and IL-1β. The tinnitus scores of salicylate-treated mice showed significant positive associations with the expression levels of the TNF-α and IL-1β, and NR2B genes. Furthermore, the gene expression levels of TNF-α and IL-1β correlated positively with that of the NR2B gene. We suggest that these proinflammatory cytokines might lead to tinnitus directly or via modulation of NR2B gene expression in salicylate-induced tinnitus.

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**Authors’ contributions**

JHH conceived and designed the study. SYY carried out the laboratory experiments. JHH and YCC performed the statistical analysis. JHH drafted the manuscript. JCC, MFW and YCC supervised the study. All authors participated in the critical revision of the manuscript. All authors have given final approval of the manuscript to be published.

**Competing interests**

The authors declare that they have no competing interests.

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**References**

1. Jastreboff PJ, Brennan J, Coleman JK, Sasaki CT: Phantom auditory sensation in rats: an animal model for tinnitus. Behav Neurosi 1988, 102:611-622.

2. Gatttoni MA, Caston J, Ruehl J, Johnson RM, Pujol R, Puel JL: Salicylate induces tinnitus through activation of cochlear NMDA receptors. J Neurosci 2003, 23:3944-3952.

3. Im GJ, Jung HH, Choe SW, Cho WS, Kim SJ: Differential gene expression profiles in salicylate ototoxicity of the mouse. Acta Otolaryngol 2007, 127:459-469.

4. Jia MH, Qin ZB: Expression of c-fos and NR2A in auditory cortex of rats experienced tinnitus. Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 2006, 41:451-454. [Article in Chinese].

5. Hwang JH, Chen JC, Yang SY, Wang MF, Liu TC, Chan YC: Expression of COX-2 and NMDA receptor genes at the cochlea and midbrain in salicylate-induced tinnitus. Laryngoscope 2011, 121:361-364.

6. Fujoka M, Kanzaki S, Okano H, Masuda M, Ogawa K, Okano H: Proinflammatory cytokines expression in noise-induced damaged cochlea. J Neurosci Res 2008, 83:575-583.

7. Park HJ, Kim HJ, Bae GS, Seo SW, Kim DY, Jung WS, Kim MS, Song MY, Kim EK, Kwon KB, Hwang SY, Song HJ, Park CS, Park BK, Chong MS, Park SJ: Selective GSK-3beta inhibitors attenuate the cisplatin-induced cytotoxicity of auditory cells. Hear Res 2009, 257:53-62.

8. Weber C, Arck P, Mazurek B, Klapp BF: Impact of a relaxation training on psychometric and immunologic parameters in tinnitus sufferers. J Physiol Pharmacol 2002, 52:29-33.

9. Wheeler D, Knapp E, Bandaru VV, Wang Y, Knorr D, Poirier C, Mattson MP, Geiger JD, Haughey NJ: Tumor necrosis factor-alpha-induced neutral sphingomyelinase-2 modulates synaptic plasticity by controlling the membrane insertion of NMDA receptors. J Neurosci 2009, 109:1237-1249.

10. Wu HM, Tseng NS, Qian L, Wei SJ, Hu X, Chen SH, Rawles SM, Flood P, Hong JS, Lu RB: Novel neuroprotective mechanisms of memantine: increase in neurotrophic factor release from astroglia and anti-inflammation by preventing microglial activation. Neuropsychopharmacology 2009, 34:2944-57.

11. Zhang R, Sun L, Hayashi Y, Liu X, Koyama S, Wu Z, Nakanishi H: Acute p38-mediated inhibition of NMDA-induced outward currents in hippocampal CA1 neurons by interleukin-1beta. Neurobiol Dis 2010, 38:68-77.

12. Mcleanreay TA, Ma Y, Chen Y, Taglalatela G, Yin H, Zhang WR, Westlund KN: A peripheral neuroimmune link: gluatamate agonists upregulate NMDA NR1 receptor mRNA and protein, vimentin, TNF-alpha, and RANTES in cultured human synoviocytes. Am J Physiol Regul Integr Comp Physiol 2010, 298:R584-88.

13. Zhang RX, Li A, Liu B, Wang L, Ren K, Zhang H, Berman BM, Lao L: IL-1ra alleviates inflammatory hyperalgesia through preventing phosphorylation of NMDA receptor NR-1 subunit in rats. Pain 2008, 135:232-239.

14. Zhang RX, Li A, Liu B, Wang L, Ren K, Qiao JT, Berman BM, Lao L: Interleukin 1beta facilitates bone cancer pain in rats by enhancing NMDA receptor NR-1 subunit phosphorylation. Neuroscience 2008, 154:1533-1538.

15. Balosso S, Ravizza T, Pierucci M, Calcagno E, Invernizzi R, Di Giovanni G, Esposto E, Vezzani A: Molecular and functional interactions between tumor necrosis factor-alpha receptors and the glutamatergic system in the mouse hippocampus: implications for seizure susceptibility. Neurosci 2009, 161:299-302.

16. Nakazawa T: Mechanism of N-methyl-D-aspartate-induced retinal ganglion cell death. Nippon Ganka Gakkai Zasshi 2009, 113:1060-1070, [Article in Japanese].

17. Han P, Whelan PJ: Tumor necrosis factor alpha enhances glutamatergic transmission onto spinal motoneurons. J Neurotrauma 2010, 27:287-292.

18. Evans ES, Bovense TA: Otoxic effects of salicylates on the responses of single cochlear nerve fibres and on cochlear potentials. Br J Audiol 1982, 16:101-108.

19. Paul AK, Lobarinza E, Simmons R, Wake D, Luisi JC, Spernyak J, Mazurchuk R, Geiger JD, Haughey NJ: Macrophage inflammatory proteins in cytomegalovirus-related inner ear injury. Otol Neurotol 2000, 21:101-108.

20. Schraff SA, Schleiss MR, Brown DK, Meinen-Derr J, Choi KY, Greenwald JH, Choo DK: Macrophage inflammatory proteins in cytomegalovirus-related inner ear injury. Otolaryngol Head Neck Surg 2007, 137:612-618.

21. Ghaeni BA, Kempton JB, Pillers DA, Trune DR: Cochlear cytokine gene expression in murine acute otitis media. Laryngoscope 2007, 117:22-29.

22. Wakabayashi K, Fujoka M, Kanzaki S, Okano HJ, Shibata S, Yamashita D, Masuda M, Mihara M, Ohsugi Y, Ogawa K, Okano H: Blockade of interleukin-6 signaling suppressed cochlear inflammatory response and improved hearing impairment in noise-damaged mice cochlea. J Neurochem 2010, 56:432-443.

23. Grisham MB, Grisham WC: Tumor necrosis factor-alpha can induce recruitment of inflammatory cells to the cochlea. Otol Neurotol 2008, 29:854-859.

24. Folmer RL, Griest SE, Martin WH: Chronic tinnitus as phantom auditory pain. Otolaryngol Head Neck Surg 2001, 124:394-400.

25. Reuvekamp A, Patel S, Fox A, Walker K, Urban L: Intrathecally administered endotoxin or cytokines produce allostody, hyperalgesia and changes in
spinal cord neuronal responses to nociceptive stimuli in the rat. *Eur J Pain* 2000, 4:247-257.

27. Dahl E, Cohen SP: Perineural injection of etanercept as a treatment for postamputation pain. *Clin J Pain* 2008, 24:172-175.

28. Gustafson-Vickers SL, Lu VB, Lai AW, Todd KG, Ballanyi K, Smith PA: Long-term actions of interleukin-1beta on delay and tonic firing neurons in rat superficial dorsal horn and their relevance to central sensitization. *Mol Pain* 2008, 4:63.

29. Kawasaki Y, Zhang L, Cheng JK, Ji RR: Cytokine mechanisms of central sensitization: distinct and overlapping role of interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha in regulating synaptic and neuronal activity in the superficial spinal cord. *J Neurosci* 2008, 28:5189-5194.

30. Rothman SM, Huang Z, Lee KE, Weisshaar CL, Winkelstein BA: Cytokine mRNA expression in painful radiculopathy. *J Pain* 2009, 10:90-99.

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